(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 November 2001 (01.11.2001)

PCT

(10) International Publication Number
WO 01/81418 A1

(51) International Patent Classification[7]: C07K 14/72, C12N 15/16

(21) International Application Number: PCT/US01/13386

(22) International Filing Date: 25 April 2001 (25.04.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/200,718 27 April 2000 (27.04.2000) US

(71) Applicant *(for all designated States except US)*: **MERCK & CO., INC.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LIU, Qingyun** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **LYNCH, Kevin, R.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **HOWARD, Andrew, D.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **MELLIN, Theodore, N.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07095-0907 (US). **STRACK, Alison** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07095-0907 (US). **VAN DER PLOEG, Leonardus, H., T.** [NL/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **WANG, Ruiping** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **JIANG, Qingping** [CN/US]; 126 East Lincoln Avenue, Rahway, NJ 07095-0907 (US). **WILLIAMS, David** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(74) Common Representative: **MERCK & CO., INC.**; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(81) Designated States *(national)*: CA, JP, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NEW NEUROMEDIN U RECEPTOR NMUR2 AND NUCLEOTIDES ENCODING IT

(57) Abstract: A new neuromedin U receptor, designated NMUR2 has been found, which is involved in modulation of feeding behavior in mammals. Ligands of this receptor are able to modulate eating, and weight gain. Amino acid sequences of the human and rat forms, as well as their nucleic acid sequences are given.

WO 01/81418 A1

NEW NEUROMEDIN U RECEPTOR NMUR2 AND NUCLEOTIDES ENCODING IT

FIELD OF THE INVENTION

This invention relates to new human and rat neuromedin U receptors, designated hNMUR2, and rNMUR2, to nucleic acids encoding them, and to use of them in various assays.

BACKGROUND OF THE INVENTION

Neuromedin U (NMU) is a neuropeptide that is widely distributed in the gut and central nervous system, particularly in brain regions implicated in the control of feeding behavior. NMU belongs to the broad class of neuropeptides first isolated from porcine spinal cord and later from other species with potent activity on smooth muscle. One orphan receptor designated FM-3 (now NMUR1) was previously identified as a high affinity receptor of NMU, which is the subject of U.S. Provisional Patent Application Serial Number 60/092,623 (filed July 13, 1998) and International Patent Application No. PCT/US99/15941 (filed July 13, 1999). NMU, when injected into the rat brain, caused a marked suppression of food intake. Thus it appears that ligands of neuromedin receptors have potential as drugs which modulate feeding and regulate weight. However, it is equally clear that NMUR1 is not the only receptor whose activity is responsible for eating behaviors.

It would be desirable to further identify and characterize other receptors whose ligands are potential drugs for eating disorders.

DETAILED DESCRIPTION OF THE INVENTION

One aspect of this invention is a novel human receptor, designated hNMUR2 (SEQ.ID.NO. 2), free from associated proteins. This invention also relates to various functional domains of this receptor, such as the extracellular domain and the intracellular domain, and to hybrid molecules comprising at least one of these sequences. Also part of this invention are nucleic acids which encode this receptor, vectors such as viral vectors, plasmids and the like, which comprise these nucleic acid

sequences, and host cells which comprise the vectors. In preferred embodiments, the nucleic acid is DNA, and especially cDNA.

Another aspect of this invention a method to identify compounds which modulate the feeding activity of a mammal comprising:

contacting the compound and a NMUR2 receptor; and

determining if activity of the NMUR2 receptor is modulated.

Another aspect of this invention is the rat homologue of the human receptor (designated rNMUR2), which is free from associated proteins (SEQ ID.NO. 6.) . This invention also relates to various functional domains of this receptor, such as the extracellular domain and the intracellular domain, and to hybrid molecules comprising at least one of these sequences. Another aspect of this invention is a nucleic acid which encodes the rNMUR2 receptor; in preferred embodiments the nucleic acid is DNA, and is preferably cDNA. Yet another aspect of this invention are vectors, such as plasmids, viral vectors, and the like which comprise a rNMUR2 gene. Still another aspect of this invention are host cells which comprise a vector carrying a rNMUR2 gene.

DESCRIPTION OF THE FIGURES

FIGURE 1 is the cDNA sequence of human NMUR2 (SEQ.ID.NO. 1).

FIGURE 2 is the predicted polypeptide sequence of human NMUR2 (SEQ.ID.NO. 2).

FIGURE 3 is the translation of the open reading frame of human NMUR2 (SEQ.ID.NOS. 3 and 4).

FIGURE 4 is the cDNA sequence of rat NMUR2 (SEQ.ID.NO. 5)

FIGURE 5 is the predicted polypeptide sequence of rat NMUR2 (SEQ.ID.NO. 6).

FIGURE 6 is the translation of the open reading frame of rat NMUR2 (SEQ.ID.NOS. 7 and 8).

FIGURE 7 is the amino acid sequences and alignments of human, rat and porcine neuromedin U (SEQ.ID.NOS. 9, 10, 11, and 12)

FIGURE 8 shows the alignment of human NMUR2 protein and rat NMR2 protein.

FIGURES 9A and 9B show functional activation of NMUR2 by NMU. FIGURE 9A is NMUR2 in the aequorin assay using HEK293/aeq17 cells transiently transfected with human NMUR2. FIGURE 9B is NMUR2 in the FLIPR assay using COS-7 cells transiently transfected with human NMUR2. In the FLIPR assay, total fluorescence was normalized to the maximum amount of fluorescence detected in the presence of the calcium ionophore A23187. (▼) porcine NMU-8; (■) human NMU-25; (▲) rat NMU-23; (♦) porcine NMU-25. All the assays are shown as the means (+/- SEM) of triplicate determinations.

FIGURES 10A and 10B are *in situ* hybridization analysis of NMUR2 in the rat brain using ^{33}P-labeled anti-sense oligonucleotide probe specific for rat NMUR2, showing specific expression of NMUR2 in the PVN (paraventricular nucleus of the hypothalamus), Ep (ependymal layer in the wall of the third ventricle), and CA1 layer of the hippocampus. The signals were completely blocked in the presence of 100-fold molar excess of unlabeled probe.

FIGURES 11A and 11B are *in situ* hybridization analysis of NMU in the rat brain. FIGURE 11A shows localization of NMU mRNA in coronal brain sections using ^{33}P-labeled anti-sense oligonucleotide probe specific for the gene encoding NMU. ARC: arcuate nucleus; ME: median eminence. The signals were completely blocked in the presence of 100-fold molar excess of unlabeled probe. FIGURE 11B shows a decrease of NMU mRNA in the ventromedial hypothalamic area in rats fasted for 48 hours. Data shown are means (± SEM) of three experiments. * $P < 0.05$, student t test.

FIGURES 12A-F show the effect of ICV-administrated NMU on food intake and other behaviors in rats. FIGURE 12A shows the effect on overnight food intake. Food intake, expressed as percentage of control group, was significantly decreased in rats injected with 3 μg (-38 ± 6%, n = 12 per group) and 10 μg (-32 ± 3%, n = 12 per group) of NMU (ANOVA, F(3) 8.4, $P = 0.0002$), and in rats injected with the positive control melanocortin agonist MT-II (0.3 μg; t(28)10.2, $P < 0.01$). ** Scheffe post hoc analysis, $P < 0.01$. FIGURE 12B shows effect on cumulative feeding duration. Feeding duration was significantly decreased in rats injected with NMU either at 3 μg (-33%) or 10 μg (-39%) or with the positive control MT-II (-71%). FIGURE 12C is core temperature change. A transient increase in core temperature was seen in the 3 μg NMU group that started about 40 min. post-dosing and lasted for approximately one hour. FIGURE 12D is change in gross motor

activity in rats in the fist hour post dosing. Activity was measured for 24 hours after NMU administration and compared to those of the same period of the pre-treatment. Gross motor activity was increased only in the first hour post-dosing and then returned to their pre-treatment levels in rats injected with either 1 or 3 μg of NMU. **, $P < 0.02$. FIGURE 12E is taste aversion. NMU at either 3 or 10 μg did not decrease saccharin intake relative to total intake at 24 hours post-dosing in a conditioned taste aversion assay. LiCl, an emetic control, decreased saccharin intake. [t test: t(6) 3.2, **, $P = 0.02$]. FIGURE 12F is sodium appetite. NMU at either 3 or 10 μg did not significantly change the total amount of salt intake while LiCl significantly decreased salt intake. [t test: t(4) 5.0, **, $P = 0.008$].

FIGURE 13 shows the various domains of human NMUR2 (SEQ.ID.NO. 2). The seven transmembrane domains (TM 1-7) are underlined. The sequence upstream of TM-1 is an extracellular domain, while sequences downstream of TM-7 is an intracellular domain.

FIGURE 14 shows the various domains of rat NMUR2 (SEQ.ID.NO. 6). The seven transmembrane domains (TM 1-7) are underlined. The sequence upstream of TM-1 is an extracellular domain, while sequences downstream of TM-7 is an intracellular domain.

As used within the specification and claims the following definitions apply:

FM-3 (also designated NMUR1) is a previously identified human neuromedin U receptor, subject of U.S. Provisional Patent Application Serial Number 60/092,623 (filed July 13, 1998) and International Patent Application No. PCT/US99/15941 (filed July 13, 1999).

NMUR2 (also designated FM-4) is a second neuromedin U receptor which plays a role in modulating the feeding behavior of a mammal. As used throughout "NMUR2" is not meant to refer to any particular origin of the NMUR2. "hNMUR2" means human NMUR2; "rNMUR2" means rat NMUR2.

NMU means neuromedin U.

"Free from associated protein" means that the receptor is not a naturally occurring NMUR2 receptor bound to its natural cell membrane.

A gene sequence and deduced amino acid sequence of a human orphan receptor was disclosed in WO 99/55732, published November 4, 1999 (assigned to

Astra Pharma, Inc.), and hereby incorporated by reference. Based on its structural similarity to the neurotensin receptor, this orphan receptor was designated NLR (neurotensin-like receptor), and it was hypothesized that its ligands would be useful agents for producing anesthesia and analgesia. The receptors of this invention share some gross structural similarity to the NLR receptor –both are the same length, but the human NMUR2 has six amino acids which differ from the NLR receptor:

Amino Acid Position	NLR	NMUR2
271	Leucine	Phenylalanine
298	Threonine	Serine
315	Leucine	Phenylalanine
371	Serine	Phenylalanine
383	Leucine	Proline
388	Valine	Methionine

These six amino acid differences may contribute to NMUR2's different activity. NMUR2 is involved with modulation of feeding behavior rather than anesthesia and analgesia.

Thus, one aspect of this invention is a method for identifying a compound which modulates feeding activity or weight of a mammal comprising:

a) contacting a cell comprising NMUR2 with the compound;

b) determining if the compound modulates NMUR2 activity.

Preferably the NMUR2 is recombinantly expressed in the cell. It may be introduced into the cell by conventional genetic engineering techniques, such as by conventional vectors including plasmids. Alternatively a cell line may be created which expresses NMUR2 in a non-transient fashion. Any host cell which is convenient may be used in these assays, preferably a human cell when the NMUR2 is the human NMUR2. Examples of suitable cell lines include 293 cells.

NMUR2 activity modulation can be determined in a number of ways. It may be a qualitative determination, i.e. a "positive" verses "negative" response. Alternately, the modulation can be quantified. Control systems may also be used, such as cells which are either mock-transfected and exposed to the putative ligand, or NMUR2 transfected cells which are exposed to a known negative or positive ligand.

In general, modulation of a receptor activity may be determined using a transactivation assay. In this assay, a "reporter construct" is introduced into a cell, which expresses either a recombinant receptor, or an endogenous receptor. The

reporter construct comprises a reporter gene encoding a protein whose transcription and/or translation is easily measured, including such genes as β-galactosidase, luciferase, aequolorin, CAT, and the like. Upstream is a promoter (either the promoter naturally associated with the reporter gene, or a heterologous promoter) and upstream of the promoter is an activation sequence. When a ligand binds to the receptor, a cascade of intracellular reactions occur, and the result is that a binding protein binds to the activation sequence, activating the promoter, and transcription and translation of the reporter gene occurs. Such assays are described in U.S. 5,401,629, which is hereby incorporated by reference.

The cell line used in this assay is preferably a mammalian cell line, more preferably a human cell line. In one preferred embodiment the cell line is HEK293/aeq17, a human embryonic kidney cell line which contains an aqueorlin reporter gene. It is described in Button et al 1993 *Cell Calcium*14:663-671, which is hereby incorporated by reference.

Another assay which is part of this invention is a FLIPR (Fluorometric Imaging Plate Reader) assay which monitors changes of intracellular Ca^{2+} concentration in real time. Thus another aspect of this invention is a method of identifying compounds which modulate the feeding behavior of an individual comprising: contacting cells expressing NMUR2 receptors with a compound; and determining changes in intracellular Ca^{+2} concentration. In these assays, human, porcine and rat NMU activated NMUR2 with high affinity, and lead to Ca^{+2} mobilization.

Another assay contemplated by this invention is a method of identifying compounds which modulate feeding behavior in an individual by a) contacting the compound and a NMUR2, and determining if binding occurs. In these assays, whole cells expressing the NMUR2 receptor are not necessary. While they can be used, membrane preparations, lysed cells or any other preparation containing receptors will suffice. Binding may be determined by monitoring behavior of a labeled ligand, such as ^{125}I-NMU-23 or appropriately labeled compound.

Rat NMUR2

Another aspect of this invention is the rat homologue of human NMUR2, and nucleic acids encoding this sequence. Rat NMUR2 was isolated using degenerate PCR on rat genomic DNA followed by genomic walking and PCR from

rat cDNA. The rat gene was identified in genomic DNA. The open reading frame of rat NMUR2 encodes a protein of 395 amino acids, and is approximately 80% identical to the human NMUR2. The rNMUR2 can be used in assays in the same was as hNMUR2.

Another aspect of this invention are active fragments of NMUR2. These proteins are G-coupled proteins, exhibiting the classic 7-transmembrane domain structure (see FIGURES 13 and 14). Thus this invention includes active fragments, such as the extracellular domain which contains the binding region, which may, alone be used in binding assays for ligands, or which may be coupled to at least one domain from another receptor, creating a hybrid receptor. Additionally hybrid receptors can be created which utilize the intracellular domain on NMUR2 and at least one other region from a different receptor. Hybrids between the rat/human sequences are also included as part of this invention.

The following non-limiting Examples are presented to better illustrate the invention.

EXAMPLES

EXAMPLE 1

Cloning of human NMUR2.

Genbank sequences were searched for sequences potentially encoding novel G protein-coupled receptors using the FAST_PAN data display tool (Retief, J.et al 1999 *Genome Res* 9:373-382, which is hereby incorporated by reference).

The genomic sequence AC008571 (Genbank accession number) contained a putative gene, preliminarily termed FM-4 that is approximately 51% identical to NMUR1 (both of which are hereby incorporated by reference).

Two primers, FM-4.F1 (5'-GAA ACA GAG CCT CGT ACC A-3') (SEQ. ID.NO. 13) and FM-4.R1 (AGT CGG ATC CAA TTC AGG TTT TGT TAA AGT GGA) (SEQ.ID.NO. 14) were synthesized and used to amplify the full-length coding sequence of FM-4 from human testis cDNA. The PCR product was cloned into the vector pCRII (Invitrogen, Inc.), sequenced, and subcloned into the mammalian expression vector pcDNA3.1(-) (Invitrogen, Inc.). It was subsequently re-named NMUR2.

EXAMPLE 2

Isolation of rat orthologs of NMUR2.

For the isolation of rat NMUR2, two degenerate primers (forward): 5'-TTC AGC CTG GCN GTN TCN GA-3' (SEQ.ID.NO. 15) and (reverse): 5'-GCT GAG GAT NGA NGC RAA RCA –3' (SEQ.ID.NO. 16) were used to carry out PCR reactions on rat genomic DNA. The resulting PCR product was subcloned into pCRII and four independent clones were sequenced. Specific primers were synthesized and used to carry out genomic walking. Sequences corresponding to the start and stop codons of human NMUR2 were identified, and PCR primers flanking the coding sequence were used to amplify the full-length open reading from rat stomach cDNA. The PCR product was cloned into pCRII and sequenced.

EXAMPLE 3

Generation of NMUR2 -Expressing Cells.

The complete coding sequence of hNMUR2 was subcloned into the expression vector pIRESpuromycin (Clontech, Inc., Palo Alto, California, USA). The plasmid hFM-4/pIRESpuro was then transfected into HEK293/aeq17 cells (Button and Brownstein, 1993, *Cell Calcium*, 14:663-671) using Lipofectamine-2000 (Gaithersburg, MD, USA) and cells stable expressing hFM-4 were selected as described in Liu et al, 1999 *Biochem. Biophys. Res. Commun.* 266:174-178, which is hereby incorporated by reference.

EXAMPLE 4

Aequorin Functional Assays

The HEK293/aeq17 cell line was licensed from NIH (Button and Brownstein, 1993, *Cell Calcium*, 14:663-671). The cells were grown in Dulbecco's Modified Medium (DMEM, GIBCO-BRL, Gaithersburg, MD, USA) + 10% fetal bovine serum (heat inactivated), 1 mM sodium pyruvate, 500 µg/ml Geneticin, 100 µg/ml streptomycin, and 100 units/ml penicillin. NMUR2 /pIRESpuro plasmid DNA was transiently transfected into HEK293/aeq17 using Lipofectamine-2000 (Gaithersburg, MD, USA) following the conditions suggested by GIBCO-BRL. Twenty four hours after transfection, cells were washed once with DMEM + 0.1 %

fetal bovine serum, and then charged for one hour at 37 °C /5% CO_2 in DMEM containing 8 μM coelenterazine cp (Molecular Probes, Eugene, OR, USA) and 30 μM glutathione. The cells were then washed once with Versene (GIBCO-BRL, Gaithersburg, MD, USA), detached using Enzyme-free cell dissociation buffer (GIBCO-BRL, Gaithersburg, MD, USA), diluted into ECB (Ham's F12 nutrient mixture (GIBCO-BRL) + 0.3 mM $CaCl_2$, 25 mM HEPES, pH7.3, 0.1% fetal bovine serum). The cell suspension was centrifuged at 500x g for 5 min. The supernatant was removed, and the pellet was then resuspended in 10 mL ECB. The cell density was determined by counting with a hemacytometer and adjusted to 500,000 cells/ml in ECB.

Human NMU-25 was custom synthesized by Research Genetics (Huntsville, AL, USA). Rat NMU-23, porcine NMU-8, and porcine NMU-25 were purchased from Phoenix Pharmaceuticals (Belmont, CA, USA). Results are shown in FIGURE 9A.

EXAMPLE 5

FLIPR Functional Assay

Cos-7 cells, grown in Dulbecco's Modified Medium (DMEM, GIBCO-BRL, Gaithersburg, MD, USA) + 10% fetal bovine serum, were transfected with h NMUR2 /pcDNA3.1 using Lipofectamine-2000 (GIBCO-BRL, Gaithersburg, MD, USA). Two days post transfection, the cells were detached and seeded into 96-well plates at approximately10,000 cells/well. The next day, cells were loaded with Fluo-3 in the presence of 2.5 mM probenicid. After washing, the cells were treated with varying concentrations of NMU. Fluorescence output was measured by a Fluorometric Imaging Plate Reader (FLIPR, Molecular Devices, Inc.). Results are shown in FIGURE 9B.

EXAMPLE 6

Expression Analysis

Quantitative *in situ* hybridization analysis in the rat brain was carried out described previously (Guan, X. M.,et al,.1998. *Brain Res Mol Brain Res* 59, 273-279 , which is hereby incorporated by reference). For rNMUR2, the probe used was ^{33}P-labeled anti-sense oligonucleotides (equal mix of oligo 420: 5'- AGG AAA GGG

TAA TTG TGC CAC ATC TCG TAG ATT TCC AGA GGC ATC -3' (SEQ.ID. NO.17) and oligo 421: 5'- CAC AGT CTC GAA GAG GGC TGT CTT GAA GTA GCA TCC CAC AGG C -3' (SEQ.ID.NO.18)). For NMU, the probe used was ^{33}P-labeled anti-sense oligonucleotide: 5'- TTC TGG TGG TAA TCT TTG AGG CGA TAT TGG CGT ACC TCT GCA AGC -3' (SEQ.ID.NO.19). Results are shown in FIGURES 10A, 10B, 11A and 11B.

EXAMPLE 7

Animal Studies

Male rats (Charles River Sprague Dawley) weighing 250-350 g were maintained in a temperature and humidity controlled facility with a 12 hour light/dark cycle (4:00AM lights on). Rats were individually housed in custom designed shoebox cages on wire floors and fed *ad libitum* with fresh diet provided daily. The shoebox cage had an external, restricted access feeder assembly that allows the animal to place only its head through an opening in the feeder assembly to access a detachable clear plastic food drawer. Attached to the food drawers was an infrared feeding monitor that projects a beam across the drawer above the food (MiniMitter, Inc., Sun River, OR). When the animal broke the infrared beam it caused a switch closure. An oscillator then sent off pulses (one pulse/second) and the total number of pulses indicated the length of time that the beam was broken which corresponds to the length of time spent feeding (recorded as feeding duration).

Cannulation and ICV administration were performed essentially as described in Murphy et al 1998 *Neuropeptides* 32:491-497, which is hereby incorporated by reference. After cannulation, rats were allowed to recover a minimum of seven days before injection with test compounds. All test substances were dissolved in artificial cerebral spinal fluid (aCSF). Rats were injected ICV with 1, 3, or 10 μg of rat NMU-23 (Phoenix Pharmaceuticals). Additional rats were injected ICV with either 0.3 or 0.03 μg of MT-II (Peninsula Laboratories) as a positive control for food intake suppression (melanocortin receptor agonist). One group of rats also had a radio transmitter placed in the peritoneal cavity for measurement of core body temperature and gross motor activity (MiniMitter, Inc., Sun River, OR). Another group of ICV-cannulated rats were used in conditioned taste aversion (CTA) and sodium appetite (SA) aversion assays.

In the CTA study, rats were conditioned to two hour daily access to water, with access to water from two bottles for two hours each day for three days.

On the fourth day, rats were given 0.15% saccharin for the two hour period instead of water and saccharin consumption measured. Rats were injected NMU-23 (0, 3, or 10 μg, ICV). LiCl was used as a positive control (0.15 M; 2 ml/kg, i.p.). On the fifth day, rats were given saccharin alone for the first hour, then water was added for the remaining 23 hours. Fluid consumption was measured at 1, 2, and 24 hours post injection. Aversion was assessed as a function of drinking preferences.

In the salt appetite assay, rats were given 0.5 M NaCl salt water to drink for three days along with food and regular water. After three days, two injections of furosemide (5 mg /0.2 ml, s.c.) were given at one hour apart to sodium-deplete the rats. Rats were then returned to salt-free water and given a sodium-deficient diet. Rats actively seek to defend their internal sodium levels. Consequently, when sodium is depleted, they will avidly drink salt solutions unless ill or nauseous. Twenty-four hours following furosemide administration, rats were given NMU (0, 3, or 10 μg, ICV), or LiCl (0.15 M, 2 ml/kg, i.p.) and given water and 0.5 M NaCl to drink. Fluid consumption was measured 1, 2, and 24 hours post dosing.

Results are shown in FIGURE 12A-F. All rodent studies described were conducted in accord with rules and guidelines of the Merck Research Laboratories Institutional Animal Care and Use Committee and the "Guidelines for the Care and Use of Laboratory Animals" [DHHS Publication No. (NIH) 85-23, revised 1985].

WHAT IS CLAIMED IS

1. A neuromedin U receptor, designated NMUR2, free from associated proteins and comprising the amino acid sequence shown in SEQ.ID.NO. 2 or SEQ.ID.NO. 6.

2. A method to identify compounds which modulate the feeding activity of a mammal comprising:

(a) contacting the compound an a NMUR2 receptor; and

(b) determining if the activity of the NMR2 receptor is modulated.

3. A method according to Claim 2 wherein step (b) is a qualitative determination.

4. A method according to Claim 2 wherein step (b) is a quantitative determination.

5. A method according to Claim 2 further comprising comparing results obtained in step (b) to results obtained using a control.

6. A method according to Claim 2 wherein step (b) comprises:

measuring transcription or translation of a reporter gene whose transcription is modulated as a result of binding of the compound to the NMR2 receptor and its resultant activation.

7. A method according to Claim 6 wherein the reporter gene is selected from the group consisting of: β-galactosidase, luciferase, aequolorin, and CAT.

8. A method according to Claim 2 wherein the NMUR2 is a recombinant NMUR2 in a mammalian host cell or cell line.

9. A method according to Claim 2 wherein step (b) comprises measuring changes of intracellular calcium concentration.

10. A method of identifying compounds which modulate feeding behavior in an individual comprising:

a) contacting the compound and a NMUR2; and

b) determining if binding of the compound and NMUR2 occurs.

11. A method according to Claim 10 wherein the compound is labeled.

12. A method according to Claim 10 wherein the NMUR2 is labeled.

13. A nucleic acid encoding a NMUR2 protein.

14. A nucleic acid according to Claim 13 which is DNA.

15. A nucleic acid according to Claim 14 which is cDNA.

16. A nucleic acid which encodes the protein shown in SEQ.ID.NO. 2 or SEQ.ID.NO. 6.

17. A nucleic acid according to Claim 16 which is DNA.

18. A nucleic acid according to Claim 13 which is present in a vector.

19. A nucleic acid according to Claim 13 which is present in a plasmid.

20. A nucleic acid according to Claim 18 which is present in a host cell.

21. A nucleic acid according to Claim 19 which is present in a host cell.

22. A nucleic acid according to Claim 20 wherein the host cell is a human cell.

23. A nucleic acid according to Claim 21 wherein the host cell is a human cell.

24. An isolated polypeptide which is SEQ.ID.NO. 2 or 6.

25. An isolated polypeptide comprising an extracellular domain of the polypeptide of SEQ.ID.NO. 2 or 6.

26. A hybrid receptor molecule comprising an extracellular domain of the polypeptide of a NMUR2 receptor and at least one other domain which is heterologous.

cDNA Polynucleotide sequence of human NMUR2
(SEQ.ID.NO. 1)

```
   1 GGCTCAGCTT GAAACAGAGC CTCGTACCAG GGGAGGCTCA GGCCTTGGAT
  51 TTTAATGTCA GGGATGGAAA AACTTCAGAA TGCTTCCTGG ATCTACCAGC
 101 AGAAACTAGA AGATCCATTC CAGAAACACC TGAACAGCAC CGAGGAGTAT
 151 CTGGCCTTCC TCTGCGGACC TCGGCGCAGC CACTTCTTCC TCCCCGTGTC
 201 TGTGGTGTAT GTGCCAATTT TTGTGGTGGG GGTCATTGGC AATGTCCTGG
 251 TGTGCCTGGT GATTCTGCAG CACCAGGCTA TGAAGACGCC CACCAACTAC
 301 TACCTCTTCA GCCTGGCGGT CTCTGACCTC CTGGTCCTGC TCCTTGGAAT
 351 GCCCCTGGAG GTCTATGAGA TGTGGCGCAA CTACCCTTTC TTGTTCGGGC
 401 CCGTGGGCTG CTACTTCAAG ACGGCCCTCT TTGAGACCGT GTGCTTCGCC
 451 TCCATCCTCA GCATCACCAC CGTCAGCGTG GAGCGCTACG TGGCCATCCT
 501 ACACCCGTTC CGCGCCAAAC TGCAGAGCAC CCGGCGCCGG GCCCTCAGGA
 551 TCCTCGGCAT CGTCTGGGGC TTCTCCGTGC TCTTCTCCCT GCCCAACACC
 601 AGCATCCATG GCATCAAGTT CCACTACTTC CCCAATGGGT CCCTGGTCCC
 651 AGGTTCGGCC ACCTGTACGG TCATCAAGCC CATGTGGATC TACAATTTCA
 701 TCATCCAGGT CACCTCCTTC CTATTCTACC TCCTCCCCAT GACTGTCATC
 751 AGTGTCCTCT ACTACCTCAT GGCACTCAGA CTAAAGAAAG ACAAATCTCT
 801 TGAGGCAGAT GAAGGGAATG CAAATATTCA AAGACCCTGC AGAAAATCAG
 851 TCAACAAGAT GCTGTTTGTC TTGGTCTTAG TGTTTGCTAT CTGTTGGGCC
 901 CCGTTCCACA TTGACCGACT CTTCTTCAGC TTTGTGGAGG AGTGGAGTGA
 951 ATCCCTGGCT GCTGTGTTCA ACCTCGTCCA TGTGGTGTCA GGTGTCTTCT
1001 TCTACCTGAG CTCAGCTGTC AACCCCATTA TCTATAACCT ACTGTCTCGC
1051 CGCTTCCAGG CAGCATTCCA GAATGTGATC TCTTCTTTCC ACAAACAGTG
1101 GCACTCCCAG CATGACCCAC AGTTGCCACC TGCCCAGCGG AACATCTTCC
1151 TGACAGAATG CCACTTTGTG GAGCTGACCG AAGATATAGG TCCCCAATTC
1201 CCATGTCAGT CATCCATGCA CAACTCTCAC CTCCCAACAG CCCTCTCTAG
1251 TGAACAGATG TCAAGAACAA ACTATCAAAG CTTCCACTTT AACAAAACCT
1301 GAATTCTTTC AGAGCTGATC TCTCCTCTAT GCCTCAAAAC TTCA
```

FIG.1

Predicted polypeptide sequence of human NMUR2
(SEQ.ID.NO. 2)

1 MSGMEKLQNA SWIYQQKLED PFQKHLNSTE EYLAFLCGPR RSHFFLPVSV

51 VYVPIFVVGV IGNVLVCLVI LQHQAMKTPT NYYLFSLAVS DLLVLLLGMP

101 LEVYEMWRNY PFLFGPVGCY FKTALFETVC FASILSITTV SVERYVAILH

151 PFRAKLQSTR RRALRILGIV WGFSVLFSLP NTSIHGIKFH YFPNGSLVPG

201 SATCTVIKPM WIYNFIIQVT SFLFYLLPMT VISVLYYLMA LRLKKDKSLE

251 ADEGNANIQR PCRKSVNKML FVLVLVFAIC WAPFHIDRLF FSFVEEWSES

301 LAAVFNLVHV VSGVFFYLSS AVNPIIYNLL SRRFQAAFQN VISSFHKQWH

351 SQHDPQLPPA QRNIFLTECH FVELTEDIGP QFPCQSSMHN SHLPTALSSE

401 QMSRTNYQSF HFNKT

FIG.2

Translation of the open reading frame of human NMRU2
(SEQ.ID.NOS. 3 and 4)

```
        10                  30                  50
GGCTCAGCTTGAAACAGAGCCTCGTACCAGGGGAGGCTCAGGCCTTGGATTTTAATGTCA
                                                MetSer

        70                  90                  110
GGGATGGAAAAACTTCAGAATGCTTCCTGGATCTACCAGCAGAAACTAGAAGATCCATTC
GlyMetGluLysLeuGlnAsnAlaSerTrpIleTyrGlnGlnLysLeuGluAspProPhe

       130                 150                 170
CAGAAACACCTGAACAGCACCGAGGAGTATCTGGCCTTCCTCTGCGGACCTCGGCGCAGC
GlnLysHisLeuAsnSerThrGluGluTyrLeuAlaPheLeuCysGlyProArgArgSer

       190                 210                 230
CACTTCTTCCTCCCCGTGTCTGTGGTGTATGTGCCAATTTTTGTGGTGGGGGTCATTGGC
HisPhePheLeuProValSerValValTyrValProIlePheValValGlyValIleGly

       250                 270                 290
AATGTCCTGGTGTGCCTGGTGATTCTGCAGCACCAGGCTATGAAGACGCCCACCAACTAC
AsnValLeuValCysLeuValIleLeuGlnHisGlnAlaMetLysThrProThrAsnTyr

       310                 330                 350
TACCTCTTCAGCCTGGCGGTCTCTGACCTCCTGGTCCTGCTCCTTGGAATGCCCCTGGAG
TyrLeuPheSerLeuAlaValSerAspLeuLeuValLeuLeuLeuGlyMetProLeuGlu

       370                 390                 410
GTCTATGAGATGTGGCGCAACTACCCTTTCTTGTTCGGGCCCGTGGGCTGCTACTTCAAG
ValTyrGluMetTrpArgAsnTyrProPheLeuPheGlyProValGlyCysTyrPheLys

       430                 450                 470
ACGGCCCTCTTTGAGACCGTGTGCTTCGCCTCCATCCTCAGCATCACCACCGTCAGCGTG
ThrAlaLeuPheGluThrValCysPheAlaSerIleLeuSerIleThrThrValSerVal

       490                 510                 530
GAGCGCTACGTGGCCATCCTACACCCGTTCCGCGCCAAACTGCAGAGCACCCGGCGCCGG
GluArgTyrValAlaIleLeuHisProPheArgAlaLysLeuGlnSerThrArgArgArg

       550                 570                 590
GCCCTCAGGATCCTCGGCATCGTCTGGGGCTTCTCCGTGCTCTTCTCCCTGCCCAACACC
AlaLeuArgIleLeuGlyIleValTrpGlyPheSerValLeuPheSerLeuProAsnThr

       610                 630                 650
AGCATCCATGGCATCAAGTTCCACTACTTCCCCAATGGGTCCCTGGTCCCAGGTTCGGCC
SerIleHisGlyIleLysPheHisTyrPheProAsnGlySerLeuValProGlySerAla
```

FIG.3A

```
       670                 690                 710
ACCTGTACGGTCATCAAGCCCATGTGGATCTACAATTTCATCATCCAGGTCACCTCCTTC
ThrCysThrValIleLysProMetTrpIleTyrAsnPheIleIleGlnValThrSerPhe

       730                 750                 770
CTATTCTACCTCCTCCCCATGACTGTCATCAGTGTCCTCTACTACCTCATGGCACTCAGA
LeuPheTyrLeuLeuProMetThrValIleSerValLeuTyrTyrLeuMetAlaLeuArg

       790                 810                 830
CTAAAGAAAGACAAATCTCTTGAGGCAGATGAAGGGAATGCAAATATTCAAAGACCCTGC
LeuLysLysAspLysSerLeuGluAlaAspGluGlyAsnAlaAsnIleGlnArgProCys

       850                 870                 890
AGAAAATCAGTCAACAAGATGCTGTTTGTCTTGGTCTTAGTGTTTGCTATCTGTTGGGCC
ArgLysSerValAsnLysMetLeuPheValLeuValLeuValPheAlaIleCysTrpAla

       910                 930                 950
CCGTTCCACATTGACCGACTCTTCTTCAGCTTTGTGGAGGAGTGGAGTGAATCCCTGGCT
ProPheHisIleAspArgLeuPhePheSerPheValGluGluTrpSerGluSerLeuAla

       970                 990                1010
GCTGTGTTCAACCTCGTCCATGTGGTGTCAGGTGTCTTCTTCTACCTGAGCTCAGCTGTC
AlaValPheAsnLeuValHisValValSerGlyValPhePheTyrLeuSerSerAlaVal

      1030                1050                1070
AACCCCATTATCTATAACCTACTGTCTCGCCGCTTCCAGGCAGCATTCCAGAATGTGATC
AsnProIleIleTyrAsnLeuLeuSerArgArgPheGlnAlaAlaPheGlnAsnValIle

      1090                1110                1130
TCTTCTTTCCACAAACAGTGGCACTCCCAGCATGACCCACAGTTGCCACCTGCCCAGCGG
SerSerPheHisLysGlnTrpHisSerGlnHisAspProGlnLeuProProAlaGlnArg

      1150                1170                1190
AACATCTTCCTGACAGAATGCCACTTTGTGGAGCTGACCGAAGATATAGGTCCCCAATTC
AsnIlePheLeuThrGluCysHisPheValGluLeuThrGluAspIleGlyProGlnPhe

      1210                1230                1250
CCATGTCAGTCATCCATGCACAACTCTCACCTCCCAACAGCCCTCTCTAGTGAACAGATG
ProCysGlnSerSerMetHisAsnSerHisLeuProThrAlaLeuSerSerGluGlnMet

      1270                1290                1310
TCAAGAACAAACTATCAAAGCTTCCACTTTAACAAAACCTGAATTCTTTCAGAGCTGATC
SerArgThrAsnTyrGlnSerPheHisPheAsnLysThr*

      1330
TCTCCTCTATGCCTCAAAACTTCA
```

FIG.3B

cDNA Polynucleotide Sequence of rat NMUR2
(SEQ.ID.NO. 5)

```
   1 ATGGGAAAAC TTGAAAATGC TTCCTGGATC CACGATCCAC TCATGAAGTA
  51 CTTGAACAGC ACAGAGGAGT ACTTGGCCCA CCTGTGTGGA CCCAAGCGCA
 101 GTGACCTATC CCTTCCGGTG TCTGTGGCCT ATGCGCTGAT CTTCCTGGTG
 151 GGGGTAATGG GCAATCTTCT GGTGTGCATG GTGATTGTCC GACATCAGAC
 201 TTTGAAGACA CCCACCAACT ACTATCTCTT CAGCTTGGCA GTCTCAGATC
 251 TGCTGGTCCT GCTCTTGGGG ATGCCTCTGG AAATCTACGA GATGTGGCAC
 301 AATTACCCTT TCCTGTTCGG GCCTGTGGGA TGCTACTTCA AGACAGCCCT
 351 CTTCGAGACT GTGTGCTTTG CCTCCATTCT CAGTGTCACC ACGGTTAGCG
 401 TAGAGCGCTA TGTGGCCATT GTCCACCCTT TCCGAGCCAA GCTGGAGAGC
 451 ACGCGGCGAC GGGCCCTCAG GATCCTCAGC CTAGTCTGGA GCTTCTCTGT
 501 GGTCTTTTCT TTGCCCAATA CCAGCATCCA TGGCATCAAG TTCCAGCACT
 551 TTCCCAACGG GTCCTCCGTA CCTGGCTCAG CCACCTGCAC AGTCACCAAA
 601 CCCATGTGGG TGTATAACTT GATCATCCAA GCTACCAGCT TCCTCTTCTA
 651 CATCCTCCCA ATGACCCTCA TCAGCGTCCT CTACTACCTC ATGGGGCTCA
 701 GGCTGAAGAG AGATGAATCC CTTGAGGCGA ACAAAGTGGC TGTGAATATT
 751 CACAGACCCT CTAGAAAGTC AGTCACCAAG ATGCTGTTTG TCTTGGTCCT
 801 CGTGTTTGCC ATCTGCTGGA CCCCCTTCCA TGTGGACCGG CTCTTCTTCA
 851 GCTTTGTGGA AGAGTGGACA GAGTCCCTGG CTGCTGTGTT CAACCTCATC
 901 CATGTGGTAT CAGGTGTCTT CTTTTATCTG AGCTCCGCGG TCAACCCCAT
 951 TATCTATAAC CTCCTGTCTC GGCGCTTCCG GGCGGCCTTT CGAAATGTTG
1001 TCTCCCCTAC CTGCAAATGG TGCCATCCCC GGCATCGGCC ACAGGGACCT
1051 CCAGCCCAGA AGATCATCTT CTTGACAGAA TGTCACCTCG TGGAGCTGAC
1101 AGAGGATGCA GGCCCCCAGT TCCCTGGTCA GTCATCCATC CACAACACCA
1151 ACCTTACCAC GGCCCCCTGT GCAGGAGAGG TACCATAA
```

FIG.4

Predicted Polypeptide Sequence of rat NMUR2
SEQ.ID.NO. 6

1 MGKLENASWI HDPLMKYLNS TEEYLAHLCG PKRSDLSLPV SVAYALIFLV

51 GVMGNLLVCM VIVRHQTLKT PTNYYLFSLA VSDLLVLLLG MPLEIYEMWH

101 NYPFLFGPVG CYFKTALFET VCFASILSVT TVSVERYVAI VHPFRAKLES

151 TRRRALRILS LVWSFSVVFS LPNTSIHGIK FQHFPNGSSV PGSATCTVTK

201 PMWVYNLIIQ ATSFLFYILP MTLISVLYYL MGLRLKRDES LEANKVAVNI

251 HRPSRKSVTK MLFVLVLVFA ICWTPFHVDR LFFSFVEEWT ESLAAVFNLI

301 HVVSGVFFYL SSAVNPIIYN LLSRRFRAAF RNVVSPTCKW CHPRHRPQGP

351 PAQKIIFLTE CHLVELTEDA GPQFPGQSSI HNTNLTTAPC AGEVP

FIG.5

Translation of the open reading frame of rat NMUR2
(SEQ.ID.NOS.7 and 8)

```
        10                  30                  50
ATGGGAAAACTTGAAAATGCTTCCTGGATCCACGATCCACTCATGAAGTACTTGAACAGC
MetGlyLysLeuGluAsnAlaSerTrpIleHisAspProLeuMetLysTyrLeuAsnSer

        70                  90                  110
ACAGAGGAGTACTTGGCCCACCTGTGTGGACCCAAGCGCAGTGACCTATCCCTTCCGGTG
ThrGluGluTyrLeuAlaHisLeuCysGlyProLysArgSerAspLeuSerLeuProVal

       130                 150                 170
TCTGTGGCCTATGCGCTGATCTTCCTGGTGGGGGGTAATGGGCAATCTTCTGGTGTGCATG
SerValAlaTyrAlaLeuIlePheLeuValGlyValMetGlyAsnLeuLeuValCysMet

       190                 210                 230
GTGATTGTCCGACATCAGACTTTGAAGACACCCACCAACTACTATCTCTTCAGCTTGGCA
ValIleValArgHisGlnThrLeuLysThrProThrAsnTyrTyrLeuPheSerLeuAla

       250                 270                 290
GTCTCAGATCTGCTGGTCCTGCTCTTGGGGATGCCTCTGGAAATCTACGAGATGTGGCAC
ValSerAspLeuLeuValLeuLeuLeuGlyMetProLeuGluIleTyrGluMetTrpHis

       310                 330                 350
AATTACCCTTTCCTGTTCGGGCCTGTGGGATGCTACTTCAAGACAGCCCTCTTCGAGACT
AsnTyrProPheLeuPheGlyProValGlyCysTyrPheLysThrAlaLeuPheGluThr

       370                 390                 410
GTGTGCTTTGCCTCCATTCTCAGTGTCACCACGGTTAGCGTAGAGCGCTATGTGGCCATT
ValCysPheAlaSerIleLeuSerValThrThrValSerValGluArgTyrValAlaIle

       430                 450                 470
GTCCACCCTTTCCGAGCCAAGCTGGAGAGCACGCGGCGACGGGCCCTCAGGATCCTCAGC
ValHisProPheArgAlaLysLeuGluSerThrArgArgArgAlaLeuArgIleLeuSer

       490                 510                 530
CTAGTCTGGAGCTTCTCTGTGGTCTTTTCTTTGCCCAATACCAGCATCCATGGCATCAAG
LeuValTrpSerPheSerValValPheSerLeuProAsnThrSerIleHisGlyIleLys
```

FIG.6A

```
       550                   570                   590
TTCCAGCACTTTCCCAACGGGTCCTCCGTACCTGGCTCAGCCACCTGCACAGTCACCAAA
PheGlnHisPheProAsnGlySerSerValProGlySerAlaThrCysThrValThrLys

       610                   630                   650
CCCATGTGGGTGTATAACTTGATCATCCAAGCTACCAGCTTCCTCTTCTACATCCTCCCA
ProMetTrpValTyrAsnLeuIleIleGlnAlaThrSerPheLeuPheTyrIleLeuPro

       670                   690                   710
ATGACCCTCATCAGCGTCCTCTACTACCTCATGGGGCTCAGGCTGAAGAGAGATGAATCC
MetThrLeuIleSerValLeuTyrTyrLeuMetGlyLeuArgLeuLysArgAspGluSer

       730                   750                   770
CTTGAGGCGAACAAAGTGGCTGTGAATATTCACAGACCCTCTAGAAAGTCAGTCACCAAG
LeuGluAlaAsnLysValAlaValAsnIleHisArgProSerArgLysSerValThrLys

       790                   810                   830
ATGCTGTTTGTCTTGGTCCTCGTGTTTGCCATCTGCTGGACCCCCTTCCATGTGGACCGG
MetLeuPheValLeuValLeuValPheAlaIleCysTrpThrProPheHisValAspArg

       850                   870                   890
CTCTTCTTCAGCTTTGTGGAAGAGTGGACAGAGTCCCTGGCTGCTGTGTTCAACCTCATC
LeuPhePheSerPheValGluGluTrpThrGluSerLeuAlaAlaValPheAsnLeuIle

            910                   930                   950
CATGTGGTATCAGGTGTCTTCTTTTATCTGAGCTCCGCGGTCAACCCCATTATCTATAAC
HisValValSerGlyValPhePheTyrLeuSerSerAlaValAsnProIleIleTyrAsn

       970                   990                  1010
CTCCTGTCTCGGCGCTTCCGGGCGGCCTTTCGAAATGTTGTCTCCCCTACCTGCAAATGG
LeuLeuSerArgArgPheArgAlaAlaPheArgAsnValValSerProThrCysLysTrp

      1030                  1050                  1070
TGCCATCCCCGGCATCGGCCACAGGGACCTCCAGCCCAGAAGATCATCTTCTTGACAGAA
CysHisProArgHisArgProGlnGlyProProAlaGlnLysIleIlePheLeuThrGlu

      1090                  1110                  1130
TGTCACCTCGTGGAGCTGACAGAGGATGCAGGCCCCCAGTTCCCTGGTCAGTCATCCATC
CysHisLeuValGluLeuThrGluAspAlaGlyProGlnPheProGlyGlnSerSerIle

      1150                  1170
CACAACACCAACCTTACCACGGCCCCCTGTGCAGGAGAGGTACCATAA
HisAsnThrAsnLeuThrThrAlaProCysAlaGlyGluValProEnd
```

FIG.6B

Amino-acid sequences and alignment of
human, rat, and porcine NMU peptides
(SEQ.ID.NOS. 9,10,11,12)

human NMU-25 FRVDEEFQSPFASQSRGYFLFRPRN-NH_2
(SEQ.ID.NO. 9)

rat NMU-23 YKVNEYQGPVAPSGGFFLFRPRN-NH_2
(SEQ.ID.NO. 10)

porcine NMU-25 FKVDEEFQGPIASQVRRYFLFRPRN-NH_2
(SEQ.1D.NO. 11)

porcine NMU-8 YFLFRPRN-NH_2
(SEQ.ID.NO. 12)

FIG.7

Alignment of human and rat NMUR2 polypeptide sequences
(SEQ.ID.NOS.2 and 5)

```
                1                                                  50
human NMUR2     MEKLQNASWI YQQKLEDPFQ KHLNSTEEYL AFLCGPRRSH FFLPVSVVYV
rat NMUR2       MGKLENASWI H.....DPLM KYLNSTEEYL AHLCGPKRSD LSLPVSVAYA

               51                                                 100
human NMUR2     PIFVVGVIGN VLVCLVILQH QAMKTPTNYY LFSLAVSDLL VLLLGMPLEV
rat NMUR2       LIFLVGVMGN LLVCMVIVRH QTLKTPTNYY LFSLAVSDLL VLLLGMPLEI

              101                                                 150
human NMUR2     YEMWRNYPFL FGPVGCYFKT ALFETVCFAS ILSITTVSVE RYVAILHPFR
rat NMUR2       YEMWHNYPFL FGPVGCYFKT ALFETVCFAS ILSVTTVSVE RYVAIVHPFR

              151                                                 200
human NMUR2     AKLQSTRRRA LRILGIVWGF SVLFSLPNTS IHGIKFHYFP NGSLVPGSAT
rat NMUR2       AKLESTRRRA LRILSLVWSF SVVFSLPNTS IHGIKFQHFP NGSSVPGSAT

              201                                                 250
human NMUR2     CTVIKPMWIY NFIIQVTSFL FYLLPMTVIS VLYYLMALRL KKDKSLEADE
rat NMUR2       CTVTKPMWVY NLIIQATSFL FYILPMTLIS VLYYLMGLRL KRDESLEANK

              251                                                 300
human NMUR2     GNANIQRPCR KSVNKMLFVL VLVFAICWAP FHIDRLFFSF VEEWSESLAA
rat NMUR2       VAVNIHRPSR KSVTKMLFVL VLVFAICWTP FHVDRLFFSF VEEWTESLAA

              301                                                 350
human NMUR2     VFNLVHVVSG VFFYLSSAVN PIIYNLLSRR FQAAFQNVIS SFHKQWHSQH
rat NMUR2       VFNLIHVVSG VFFYLSSAVN PIIYNLLSRR FRAAFRNVVS PTCKWCHPRH

              351                                                 400
human NMUR2     DPQLPPAQRN IFLTECHFVE LTEDIGPQFP CQSSMHNSHL PTALSS.EQM
rat NMUR2       RPQGPPAQKI IFLTECHLVE LTEDAGPQFP GQSSIHNTNL TTAPCAGEVP

              401       413
human NMUR2     SRTNYQSFHF NKT
rat NMUR2       .......... ...
```

FIG.8

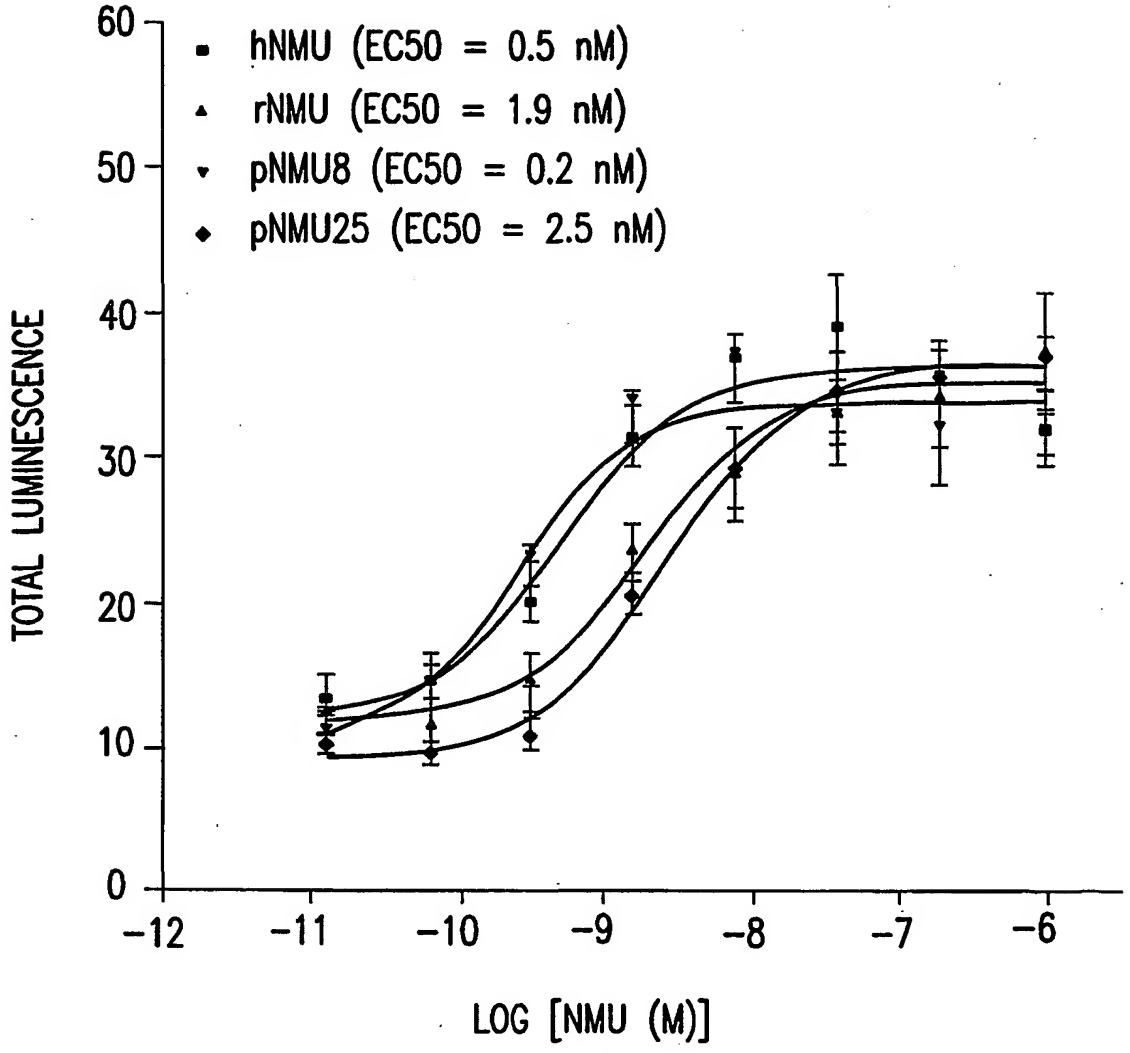
hNMU (EC50 = 0.5 nM)
rNMU (EC50 = 1.9 nM)
pNMU8 (EC50 = 0.2 nM)
pNMU25 (EC50 = 2.5 nM)
TOTAL LUMINESCENCE
60
50
40
30
20
10
0
−12
−11
−10
−9
−8
−7
−6
LOG [NMU (M)]

FIG.9A

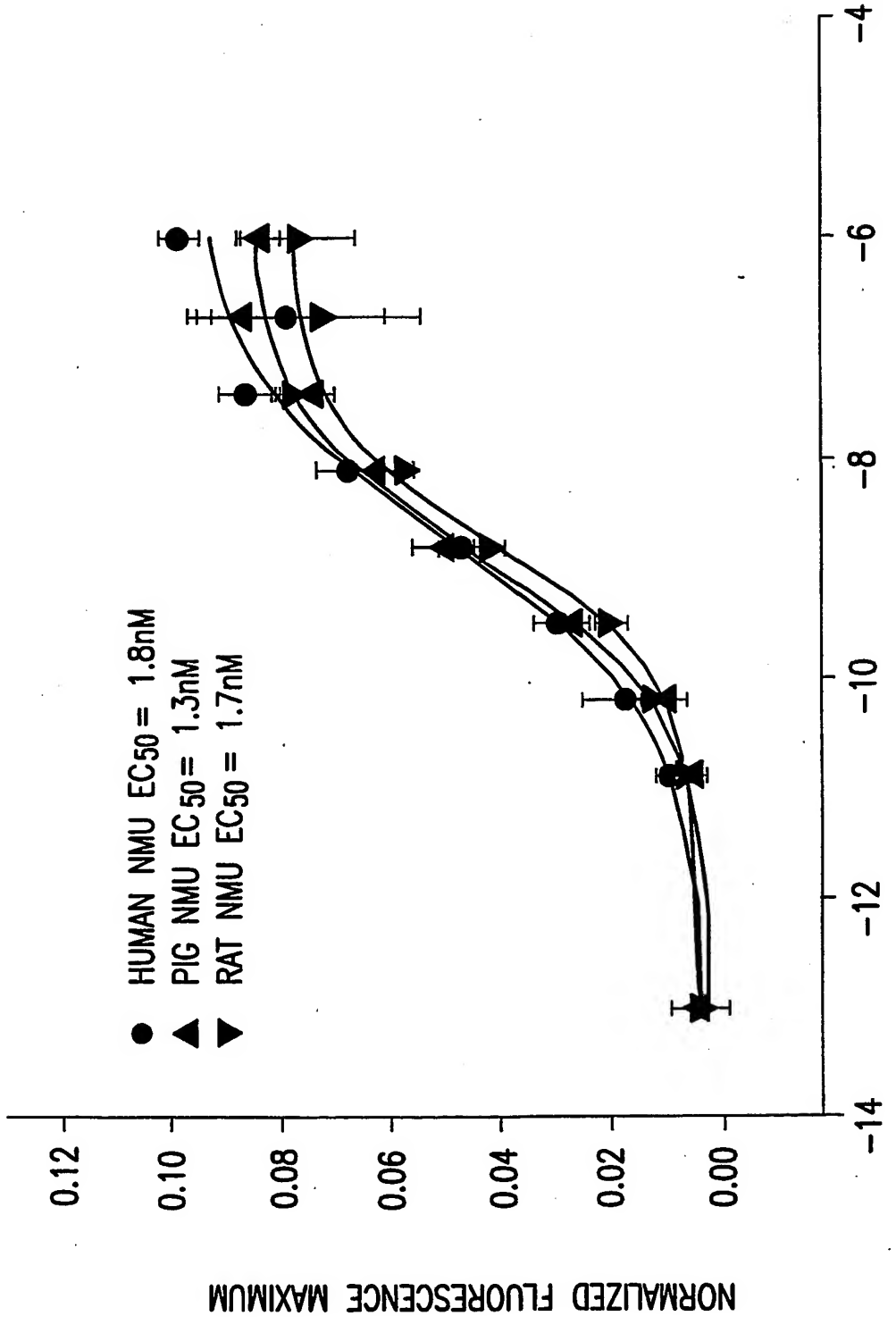
NORMALIZED FLUORESCENCE MAXIMUM
0.12
0.10
0.08
0.06
0.04
0.02
0.00
HUMAN NMU EC50 = 1.8nM
PIG NMU EC50 = 1.3nM
RAT NMU EC50 = 1.7nM
-14
-12
-10
-8
-6
-4
LOG [NEUROMEDIN U], MOLAR

FIG.9B

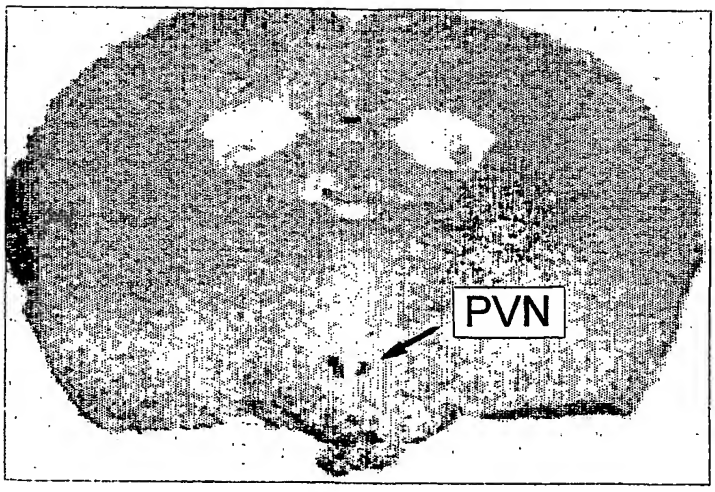

FIG.10A

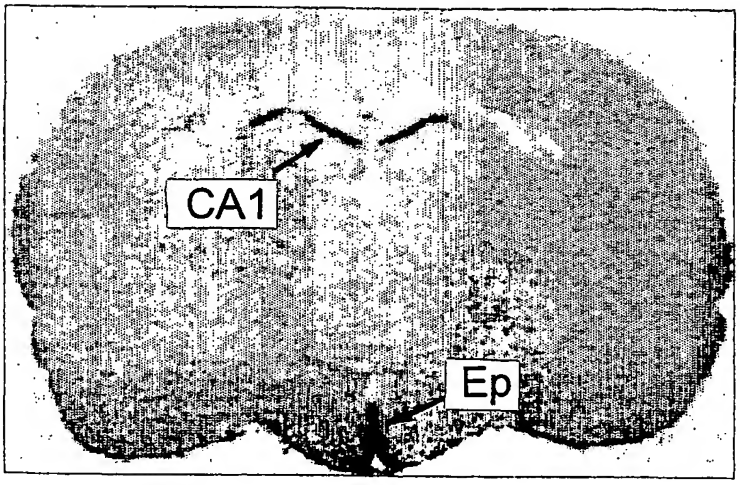

FIG.10B

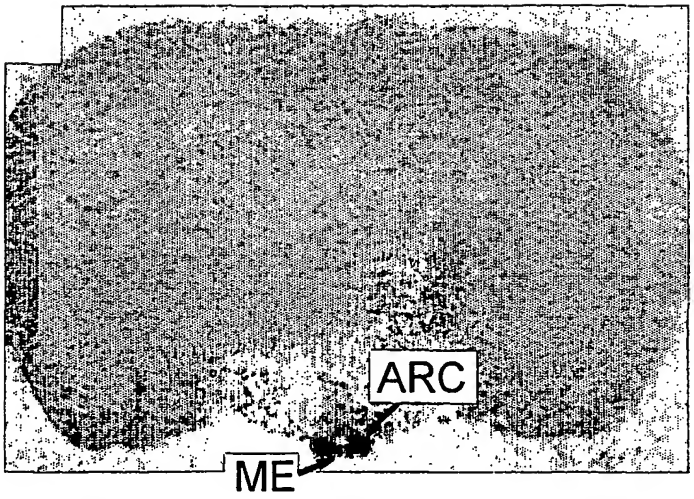

FIG.11A

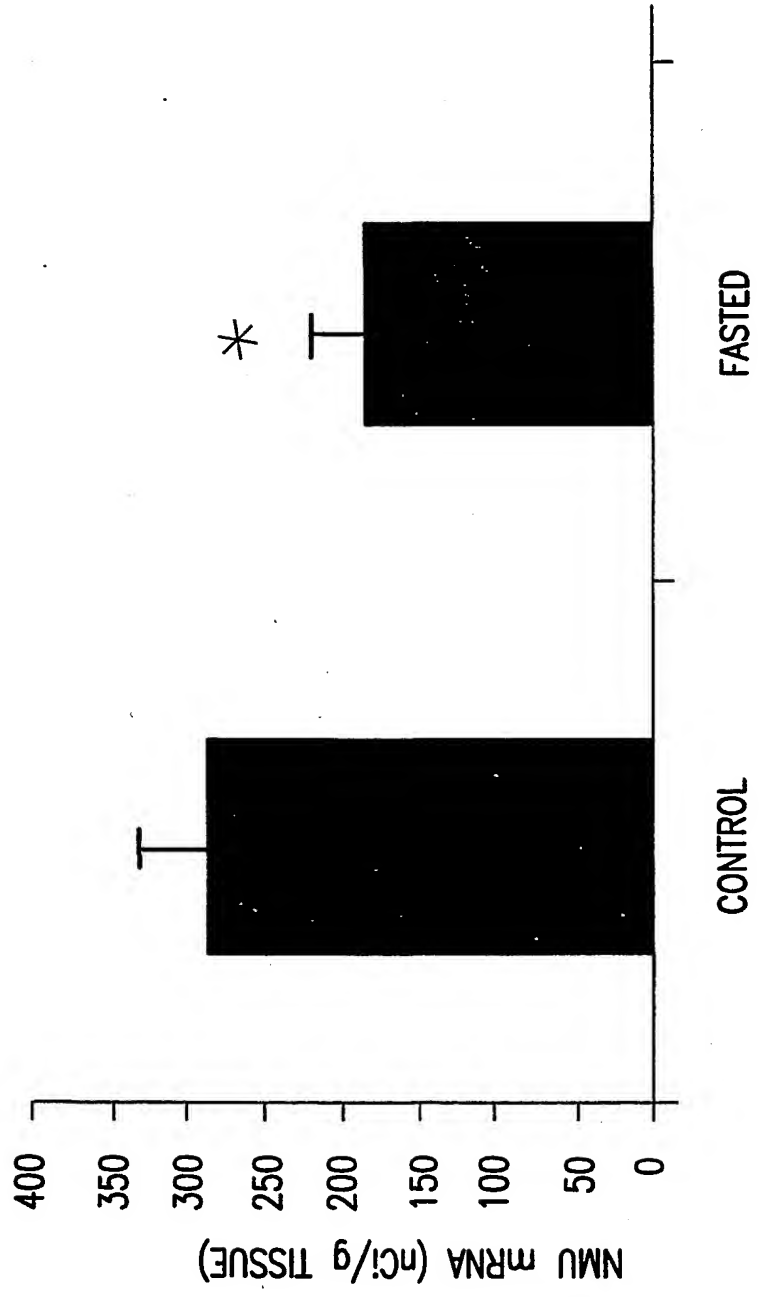
NMU mRNA (nCi/g TISSUE)
400
350
300
250
200
150
100
50
0
CONTROL
FASTED
*

FIG.11B

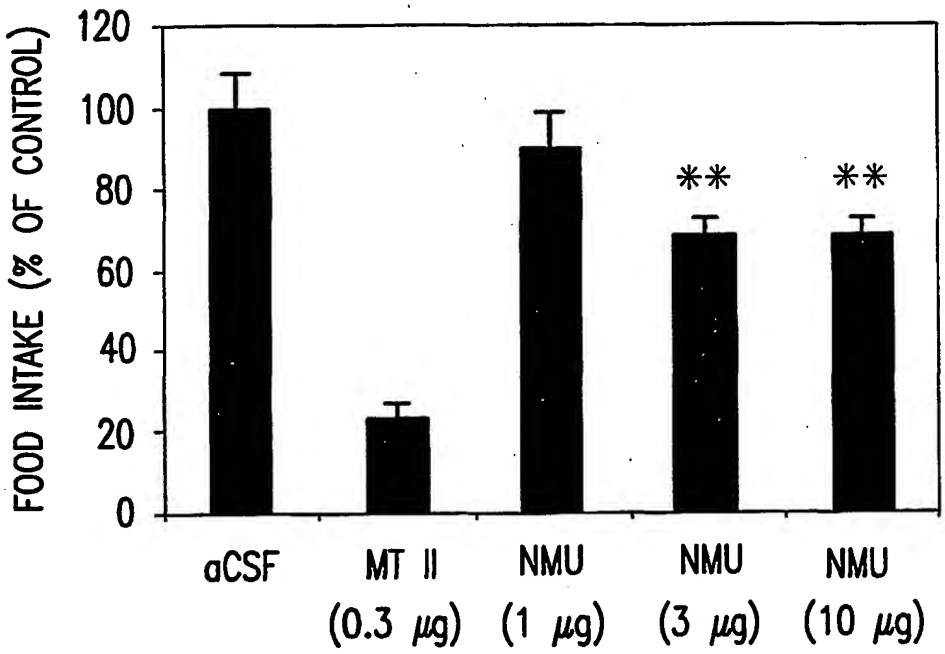

FIG.12A

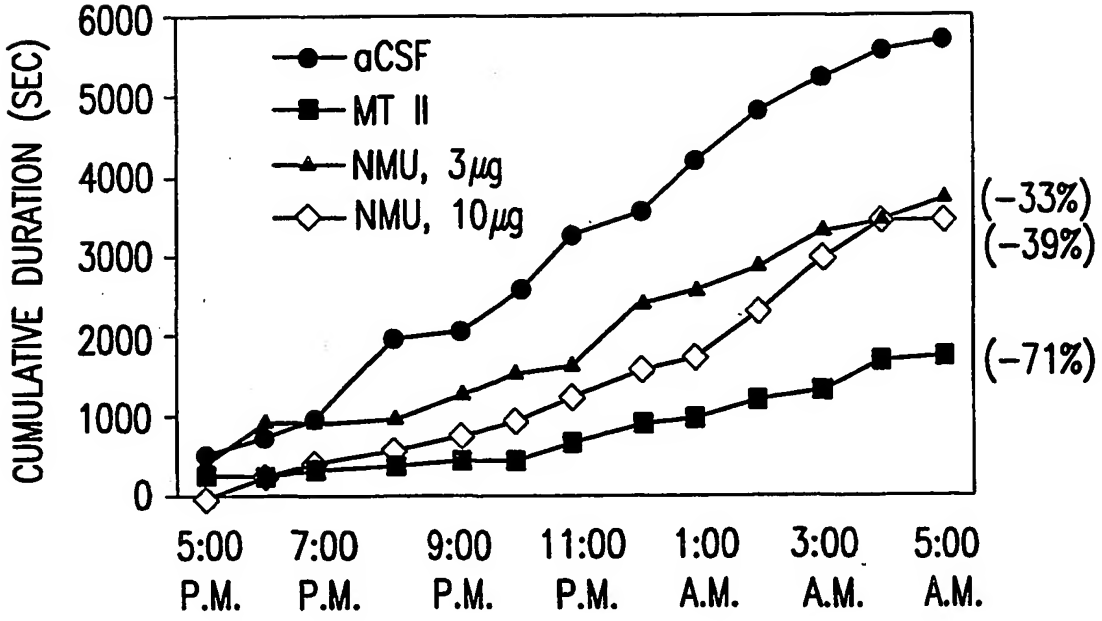

FIG.12B

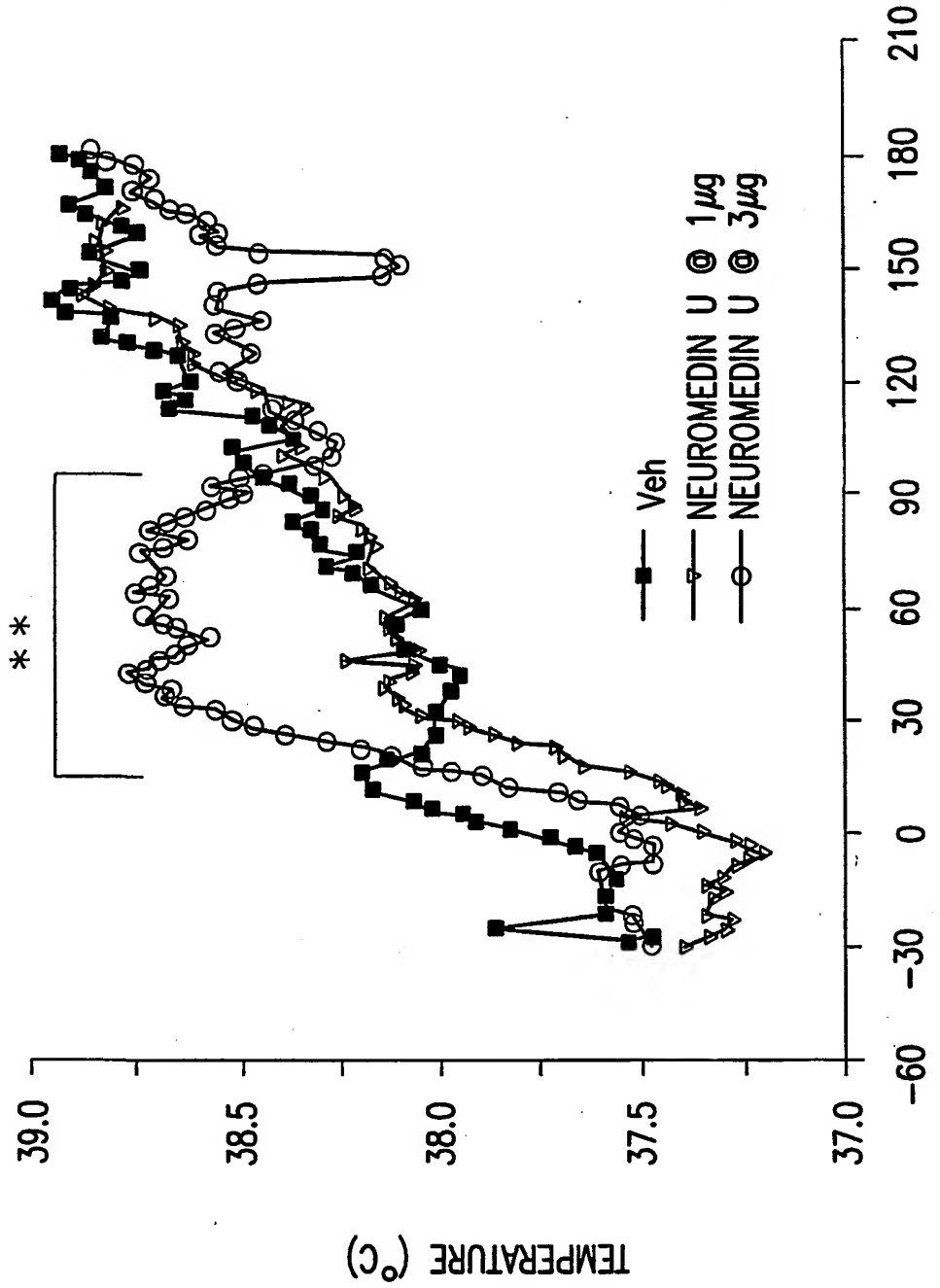
TEMPERATURE (°C)
39.0
38.5
38.0
37.5
37.0
−60
−30
0
30
60
90
120
150
180
210
TIME POST DOSE (MIN.)
Veh
NEUROMEDIN U @ 1μg
NEUROMEDIN U @ 3μg
**

FIG.12C

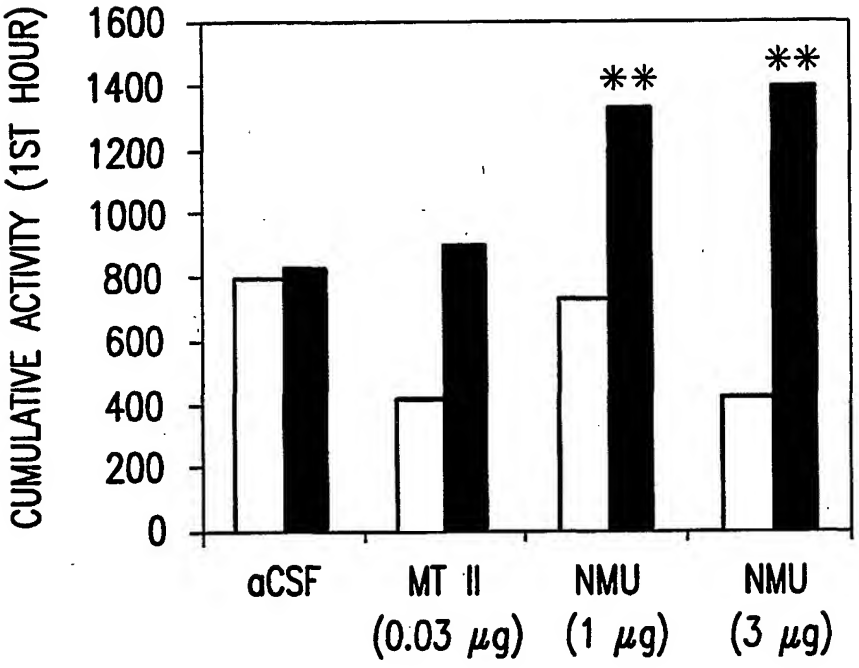
CUMULATIVE ACTIVITY (1ST HOUR)
1600
1400
1200
1000
800
600
400
200
0
**
**
aCSF
MT II (0.03 μg)
NMU (1 μg)
NMU (3 μg)

FIG.12D

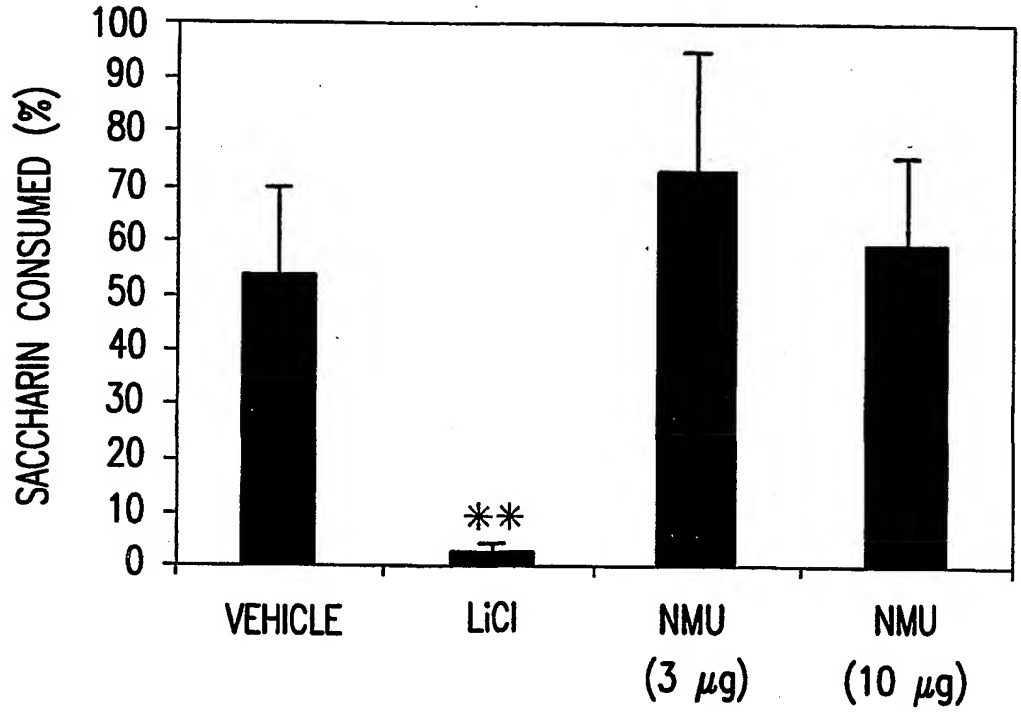

FIG.12E

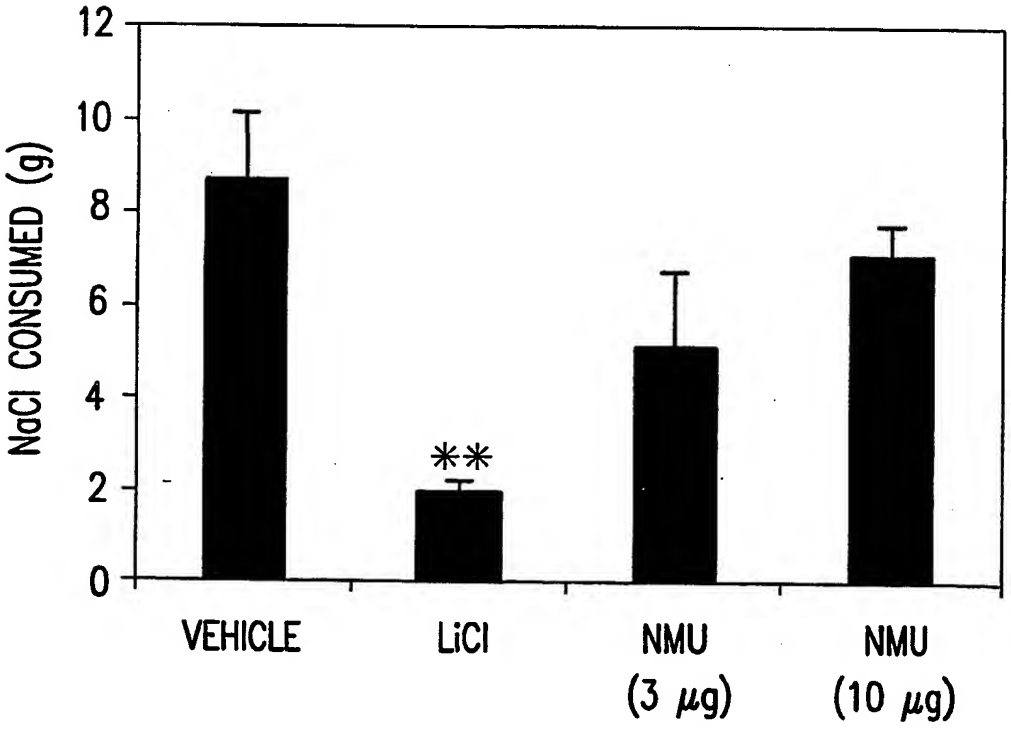

FIG.12F

Predicted polypeptide sequences of human NMUR2 and its transmembrane (TM) domain structure.

1 MSGMEKLQNA SWIYQQKLED PFQKHLNSTE EYLAFLCGPR RSHFFLPVSV

51 VYVPIFVVGV IGNVLVCLVI LQHQAMKTPT NYYLFSLAVS DLLVLLLGMP
TM-1 TM-2

101 LEVYEMWRNY PFLFGPVGCY FKTALFETVC FASILSITTV SVERYVAILH
TM-3

151 PFRAKLQSTR RRALRILGIV WGFSVLFSLP NTSIHGIKFH YFPNGSLVPG
TM-4

201 SATCTVIKPM WIYNFIIOVT SFLFYLLPMT VISVLYYLMA LRLKKDKSLE
TM-5

251 ADEGNANIQR PCRKSVNKML FVLVLVFAIC WAPFHIDRLF FSFVEEWSES
TM-6

301 LAAVFNLVHV VSGVFFYLSS AVNPIIYNLL SRRFQAAFQN VISSFHKQWH
TM-7

351 SQHDPQLPPA QRNIFLTECH FVELTEDIGP QFPCQSSMHN SHLPTALSSE

401 QMSRTNYQSF HFNKT

FIG.13

Predicted polypeptide sequences of rat NMUR2
and its transmembrane (TM) domain structure

1 MGKLENASWI HDPLMKYLNS TEEYLAHLCG PKRSDLSLPV SVAYALIFLV
TM-1

51 GVMGNLLVCM VIVRHQTLKT PTNYYLFSLA VSDLLVLLLG MPLEIYEMWH
TM-2

101 NYPFLFGPVG CYFKTALFET VCFASILSVT TVSVERYVAI VHPFRAKLES
TM-3

151 TRRRALRILS LVWSFSVVFS LPNTSIHGIK FQHFPNGSSV PGSATCTVTK
TM-4

201 PMWVYNLIIQ ATSFLFYILP MTLISVLYYL MGLRLKRDES LEANKVAVNI
TM-5

251 HRPSRKSVTK MLFVLVLVFA ICWTPFHVDR LFFSFVEEWT ESLAAVFNLI
TM-6

301 HVVSGVFFYL SSAVNPIIYN LLSRRFRAAF RNVVSPTCKW CHPRHRPQGP
TM-7

351 PAQKIIFLTE CHLVELTEDA GPQFPGQSSI HNTNLTTAPC AGEVP

FIG.14

SEQUENCE LISTING

<110> Merck & Co., Inc.
University of Virginia

<120> NEW NEUROMEDIN U RECEPTOR NMUR2 AND NUCLEOTIDES ENCODING IT

<130> 20658 PCT

<150> 60/200,718
<151> 2000-04-27

<160> 19

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 1344
<212> DNA
<213> Human

<400> 1
ggctcagctt gaaacagagc ctcgtaccag gggaggctca ggccttggat tttaatgtca 60
gggatggaaa aacttcagaa tgcttcctgg atctaccagc agaaactaga agatccattc 120
cagaaacacc tgaacagcac cgaggagtat ctggccttcc tctgcggacc tcggcgcagc 180
cacttcttcc tccccgtgtc tgtggtgtat gtgccaattt ttgtggtggg ggtcattggc 240
aatgtcctgg tgtgcctggt gattctgcag caccaggcta tgaagacgcc caccaactac 300
tacctcttca gcctggcggt ctctgacctc ctggtcctgc tccttggaat gcccctggag 360
gtctatgaga tgtggcgcaa ctacccttтc ttgttcgggc ccgtgggctg ctacttcaag 420
acggccctct ttgagaccgt gtgcttcgcc tccatcctca gcatcaccac cgtcagcgtg 480
gagcgctacg tggccatcct acacccgttc cgcgccaaac tgcagagcac ccggcgccgg 540
gccctcagga tcctcggcat cgtctggggc ttctccgtgc tcttctccct gcccaacacc 600
agcatccatg gcatcaagtt ccactacttc cccaatgggt ccctggtccc aggttcggcc 660
acctgtacgg tcatcaagcc catgtggatc tacaatttca tcatccaggt cacctccttc 720
ctattctacc tcctccccat gactgtcatc agtgtcctct actacctcat ggcactcaga 780
ctaaagaaag acaaatctct tgaggcagat gaagggaatg caaatattca aagaccctgc 840
agaaaatcag tcaacaagat gctgtttgtc ttggtcttag tgtttgctat ctgttgggcc 900
ccgttccaca ttgaccgact cttcttcagc tttgtggagg agtggagtga atccctggct 960
gctgtgttca acctcgtcca tgtggtgtca ggtgtcttct tctacctgag ctcagctgtc 1020
aaccccatta tctataacct actgtctcgc cgcttccagg cagcattcca gaatgtgatc 1080
tcttctttcc acaaacagtg gcactcccag catgacccac agttgccacc tgcccagcgg 1140
aacatcttcc tgacagaatg ccactttgtg gagctgaccg aagatatagg tccccaattc 1200
ccatgtcagt catccatgca caactctcac ctcccaacag ccctctctag tgaacagatg 1260
tcaagaacaa actatcaaag cttccacttt aacaaaacct gaattctttc agagctgatc 1320
tctcctctat gcctcaaaac ttca 1344

<210> 2
<211> 415
<212> PRT
<213> Human

<400> 2
Met Ser Gly Met Glu Lys Leu Gln Asn Ala Ser Trp Ile Tyr Gln Gln
1 5 10 15
Lys Leu Glu Asp Pro Phe Gln Lys His Leu Asn Ser Thr Glu Glu Tyr
20 25 30
Leu Ala Phe Leu Cys Gly Pro Arg Arg Ser His Phe Phe Leu Pro Val
35 40 45
Ser Val Val Tyr Val Pro Ile Phe Val Val Gly Val Ile Gly Asn Val
50 55 60

```
Leu Val Cys Leu Val Ile Leu Gln His Gln Ala Met Lys Thr Pro Thr
65                  70                  75                  80
Asn Tyr Tyr Leu Phe Ser Leu Ala Val Ser Asp Leu Leu Val Leu Leu
                85                  90                  95
Leu Gly Met Pro Leu Glu Val Tyr Glu Met Trp Arg Asn Tyr Pro Phe
            100                 105                 110
Leu Phe Gly Pro Val Gly Cys Tyr Phe Lys Thr Ala Leu Phe Glu Thr
        115                 120                 125
Val Cys Phe Ala Ser Ile Leu Ser Ile Thr Thr Val Ser Val Glu Arg
    130                 135                 140
Tyr Val Ala Ile Leu His Pro Phe Arg Ala Lys Leu Gln Ser Thr Arg
145                 150                 155                 160
Arg Arg Ala Leu Arg Ile Leu Gly Ile Val Trp Gly Phe Ser Val Leu
                165                 170                 175
Phe Ser Leu Pro Asn Thr Ser Ile His Gly Ile Lys Phe His Tyr Phe
            180                 185                 190
Pro Asn Gly Ser Leu Val Pro Gly Ser Ala Thr Cys Thr Val Ile Lys
        195                 200                 205
Pro Met Trp Ile Tyr Asn Phe Ile Ile Gln Val Thr Ser Phe Leu Phe
    210                 215                 220
Tyr Leu Leu Pro Met Thr Val Ile Ser Val Leu Tyr Tyr Leu Met Ala
225                 230                 235                 240
Leu Arg Leu Lys Lys Asp Lys Ser Leu Glu Ala Asp Glu Gly Asn Ala
                245                 250                 255
Asn Ile Gln Arg Pro Cys Arg Lys Ser Val Asn Lys Met Leu Phe Val
            260                 265                 270
Leu Val Leu Val Phe Ala Ile Cys Trp Ala Pro Phe His Ile Asp Arg
        275                 280                 285
Leu Phe Phe Ser Phe Val Glu Glu Trp Ser Glu Ser Leu Ala Ala Val
    290                 295                 300
Phe Asn Leu Val His Val Val Ser Gly Val Phe Phe Tyr Leu Ser Ser
305                 310                 315                 320
Ala Val Asn Pro Ile Ile Tyr Asn Leu Leu Ser Arg Arg Phe Gln Ala
                325                 330                 335
Ala Phe Gln Asn Val Ile Ser Ser Phe His Lys Gln Trp His Ser Gln
            340                 345                 350
His Asp Pro Gln Leu Pro Pro Ala Gln Arg Asn Ile Phe Leu Thr Glu
        355                 360                 365
Cys His Phe Val Glu Leu Thr Glu Asp Ile Gly Pro Gln Phe Pro Cys
    370                 375                 380
Gln Ser Ser Met His Asn Ser His Leu Pro Thr Ala Leu Ser Ser Glu
385                 390                 395                 400
Gln Met Ser Arg Thr Asn Tyr Gln Ser Phe His Phe Asn Lys Thr
                405                 410                 415
```

<210> 3
<211> 1344
<212> DNA
<213> Human

<400> 3

```
ggctcagctt gaaacagagc ctcgtaccag gggaggctca ggccttggat tttaatgtca      60
gggatggaaa aacttcagaa tgcttcctgg atctaccagc agaaactaga agatccattc     120
cagaaacacc tgaacagcac cgaggagtat ctggccttcc tctgcggacc tcggcgcagc     180
cacttcttcc tccccgtgtc tgtggtgtat gtgccaattt ttgtggtggg ggtcattggc     240
aatgtcctgg tgtgcctggt gattctgcag caccaggcta tgaagacgcc caccaactac     300
tacctcttca gcctggcggt ctctgacctc ctggtcctgc tccttggaat gcccctggag     360
gtctatgaga tgtggcgcaa ctacccttтc ttgttcgggc ccgtgggctg ctacttcaag     420
acggccctct ttgagaccgt gtgcttcgcc tccatcctca gcatcaccac cgtcagcgtg     480
gagcgctacg tggccatcct acacccgttc cgcgccaaac tgcagagcac ccggcgccgg     540
gccctcagga tcctcggcat cgtctggggc ttctccgtgc tcttctccct gcccaacacc     600
agcatccatg gcatcaagtt ccactacttc cccaatgggt ccctggtccc aggttcggcc     660
```

```
acctgtacgg tcatcaagcc catgtggatc tacaatttca tcatccaggt cacctccttc     720
ctattctacc tcctccccat gactgtcatc agtgtcctct actacctcat ggcactcaga     780
ctaaagaaag acaaatctct tgaggcagat gaagggaatg caaatattca aagaccctgc     840
agaaaatcag tcaacaagat gctgtttgtc ttggtcttag tgtttgctat ctgttgggcc     900
ccgttccaca ttgaccgact cttcttcagc tttgtggagg agtggagtga atccctggct     960
gctgtgttca acctcgtcca tgtggtgtca ggtgtcttct tctacctgag ctcagctgtc    1020
aaccccatta tctataacct actgtctcgc cgcttccagg cagcattcca gaatgtgatc    1080
tcttctttcc acaaacagtg gcactcccag catgacccac agttgccacc tgcccagcgg    1140
aacatcttcc tgacagaatg ccactttgtg gagctgaccg aagatatagg tccccaattc    1200
ccatgtcagt catccatgca caactctcac ctcccaacag ccctctctag tgaacagatg    1260
tcaagaacaa actatcaaag cttccacttt aacaaaacct gaattctttc agagctgatc    1320
tctcctctat gcctcaaaac ttca                                           1344
```

<210> 4
<211> 402
<212> PRT
<213> Human

<400> 4

```
Met Ser Gly Met Glu Lys Leu Gln Asn Ala Ser Trp Ile Tyr Gln Gln
 1               5                  10                  15
Lys Leu Glu Asp Pro Phe Gln Lys His Leu Asn Ser Thr Glu Glu Tyr
            20                  25                  30
Leu Ala Phe Leu Cys Gly Pro Arg Arg Ser His Phe Phe Leu Pro Val
        35                  40                  45
Ser Val Val Tyr Val Pro Ile Phe Val Val Gly Val Ile Gly Asn Val
    50                  55                  60
Leu Val Cys Leu Val Ile Leu Gln His Gln Ala Met Lys Thr Pro Thr
65                  70                  75                  80
Asn Tyr Tyr Leu Phe Ser Leu Ala Val Ser Asp Leu Leu Val Leu Leu
                85                  90                  95
Leu Gly Met Pro Leu Glu Val Tyr Glu Met Trp Arg Asn Tyr Pro Phe
            100                 105                 110
Leu Phe Gly Pro Val Gly Cys Tyr Phe Lys Thr Ala Leu Phe Glu Thr
        115                 120                 125
Val Cys Phe Ala Ser Ile Leu Ser Ile Thr Thr Val Ser Val Glu Arg
    130                 135                 140
Tyr Val Ala Ile Leu His Pro Phe Arg Ala Lys Leu Gln Ser Thr Arg
145                 150                 155                 160
Arg Arg Ala Leu Arg Ile Leu Gly Ile Val Trp Gly Phe Ser Val Leu
                165                 170                 175
Phe Ser Leu Pro Asn Thr Ser Ile His Gly Ile Lys Phe His Tyr Phe
            180                 185                 190
Pro Asn Gly Ser Leu Val Pro Gly Ser Ala Thr Cys Thr Val Ile Lys
        195                 200                 205
Pro Met Trp Ile Tyr Asn Phe Ile Ile Gln Val Thr Ser Phe Leu Phe
    210                 215                 220
Tyr Leu Leu Pro Met Thr Val Ile Ser Val Leu Tyr Tyr Leu Met Ala
225                 230                 235                 240
Leu Arg Leu Lys Lys Asp Lys Ser Leu Glu Ala Asp Glu Gly Asn Ala
                245                 250                 255
Asn Ile Gln Arg Pro Cys Arg Lys Ser Val Asn Lys Met Leu Phe Val
            260                 265                 270
Leu Val Leu Val Phe Ala Ile Cys Trp Ala Pro Phe His Ile Asp Arg
        275                 280                 285
Leu Phe Phe Ser Phe Val Glu Glu Trp Ser Glu Ser Leu Ala Ala Val
    290                 295                 300
Phe Asn Leu Val His Val Val Ser Gly Val Phe Phe Tyr Leu Ser Ser
305                 310                 315                 320
Ala Val Asn Pro Ile Ile Tyr Asn Leu Leu Ser Arg Arg Phe Gln Ala
                325                 330                 335
```

```
Ala Phe Gln Asn Val Ile Ser Ser Phe His Lys Gln Trp His Ser Gln
            340                 345                 350
His Asp Pro Gln Leu Pro Pro Ala Gln Arg Asn Ile Phe Leu Thr Glu
        355                 360                 365
Cys His Phe Val Glu Leu Thr Glu Asp Ile Gly Pro Gln Phe Pro Cys
    370                 375                 380
Gln Ser Ser Met His Asn Ser His Leu Pro Thr Ala Leu Ser Ser Glu
385                 390                 395                 400
Gln Met
```

<210> 5
<211> 1188
<212> DNA
<213> Rattus

<400> 5

```
atgggaaaac ttgaaaatgc ttcctggatc cacgatccac tcatgaagta cttgaacagc      60
acagaggagt acttggccca cctgtgtgga cccaagcgca gtgacctatc ccttccggtg     120
tctgtggcct atgcgctgat cttcctggtg ggggtaatgg gcaatcttct ggtgtgcatg     180
gtgattgtcc gacatcagac tttgaagaca cccaccaact actatctctt cagcttggca     240
gtctcagatc tgctggtcct gctcttgggg atgcctctgg aaatctacga gatgtggcac     300
aattaccctt tcctgttcgg gcctgtggga tgctacttca agacagccct cttcgagact     360
gtgtgctttg cctccattct cagtgtcacc acggttagcg tagagcgcta tgtggccatt     420
gtccaccctt tccgagccaa gctggagagc acgcggcgac gggccctcag gatcctcagc     480
ctagtctgga gcttctctgt ggtcttttct ttgcccaata ccagcatcca tggcatcaag     540
ttccagcact ttcccaacgg gtcctccgta cctggctcag ccacctgcac agtcaccaaa     600
cccatgtggg tgtataactt gatcatccaa gctaccagct tcctcttcta catcctccca     660
atgaccctca tcagcgtcct ctactacctc atggggctca ggctgaagag agatgaatcc     720
cttgaggcga acaaagtggc tgtgaatatt cacagaccct ctagaaagtc agtcaccaag     780
atgctgtttg tcttggtcct cgtgtttgcc atctgctgga ccccttcca tgtggaccgg      840
ctcttcttca gctttgtgga agagtggaca gagtccctgg ctgctgtgtt caacctcatc     900
catgtggtat caggtgtctt cttttatctg agctccgcgg tcaaccccat tatctataac     960
ctcctgtctc ggcgcttccg ggcggccttt cgaaatgttg tctcccctac ctgcaaatgg    1020
tgccatcccc ggcatcggcc acagggacct ccagcccaga agatcatctt cttgacagaa    1080
tgtcacctcg tggagctgac agaggatgca ggcccccagt tccctggtca gtcatccatc    1140
cacaacacca accttaccac ggcccccctgt gcaggagagg taccataa               1188
```

<210> 6
<211> 395
<212> PRT
<213> Rattus

<400> 6

```
Met Gly Lys Leu Glu Asn Ala Ser Trp Ile His Asp Pro Leu Met Lys
 1               5                  10                  15
Tyr Leu Asn Ser Thr Glu Glu Tyr Leu Ala His Leu Cys Gly Pro Lys
            20                  25                  30
Arg Ser Asp Leu Ser Leu Pro Val Ser Val Ala Tyr Ala Leu Ile Phe
        35                  40                  45
Leu Val Gly Val Met Gly Asn Leu Leu Val Cys Met Val Ile Val Arg
    50                  55                  60
His Gln Thr Leu Lys Thr Pro Thr Asn Tyr Tyr Leu Phe Ser Leu Ala
65                  70                  75                  80
Val Ser Asp Leu Leu Val Leu Leu Leu Gly Met Pro Leu Glu Ile Tyr
                85                  90                  95
Glu Met Trp His Asn Tyr Pro Phe Leu Phe Gly Pro Val Gly Cys Tyr
            100                 105                 110
Phe Lys Thr Ala Leu Phe Glu Thr Val Cys Phe Ala Ser Ile Leu Ser
        115                 120                 125
Val Thr Thr Val Ser Val Glu Arg Tyr Val Ala Ile Val His Pro Phe
    130                 135                 140
```

```
Arg Ala Lys Leu Glu Ser Thr Arg Arg Arg Ala Leu Arg Ile Leu Ser
145                 150                 155                 160
Leu Val Trp Ser Phe Ser Val Val Phe Ser Leu Pro Asn Thr Ser Ile
                165                 170                 175
His Gly Ile Lys Phe Gln His Phe Pro Asn Gly Ser Ser Val Pro Gly
            180                 185                 190
Ser Ala Thr Cys Thr Val Thr Lys Pro Met Trp Val Tyr Asn Leu Ile
        195                 200                 205
Ile Gln Ala Thr Ser Phe Leu Phe Tyr Ile Leu Pro Met Thr Leu Ile
    210                 215                 220
Ser Val Leu Tyr Tyr Leu Met Gly Leu Arg Leu Lys Arg Asp Glu Ser
225                 230                 235                 240
Leu Glu Ala Asn Lys Val Ala Val Asn Ile His Arg Pro Ser Arg Lys
                245                 250                 255
Ser Val Thr Lys Met Leu Phe Val Leu Val Leu Val Phe Ala Ile Cys
            260                 265                 270
Trp Thr Pro Phe His Val Asp Arg Leu Phe Phe Ser Phe Val Glu Glu
        275                 280                 285
Trp Thr Glu Ser Leu Ala Ala Val Phe Asn Leu Ile His Val Val Ser
    290                 295                 300
Gly Val Phe Phe Tyr Leu Ser Ser Ala Val Asn Pro Ile Ile Tyr Asn
305                 310                 315                 320
Leu Leu Ser Arg Arg Phe Arg Ala Ala Phe Arg Asn Val Val Ser Pro
                325                 330                 335
Thr Cys Lys Trp Cys His Pro Arg His Arg Pro Gln Gly Pro Pro Ala
            340                 345                 350
Gln Lys Ile Ile Phe Leu Thr Glu Cys His Leu Val Glu Leu Thr Glu
        355                 360                 365
Asp Ala Gly Pro Gln Phe Pro Gly Gln Ser Ser Ile His Asn Thr Asn
    370                 375                 380
Leu Thr Thr Ala Pro Cys Ala Gly Glu Val Pro
385                 390                 395
```

<210> 7
<211> 1188
<212> DNA
<213> Rattus

<400> 7

```
atgggaaaac ttgaaaatgc ttcctggatc cacgatccac tcatgaagta cttgaacagc      60
acagaggagt acttggccca cctgtgtgga cccaagcgca gtgacctatc ccttccggtg     120
tctgtggcct atgcgctgat cttcctggtg ggggtaatgg gcaatcttct ggtgtgcatg     180
gtgattgtcc gacatcagac tttgaagaca cccaccaact actatctctt cagcttggca     240
gtctcagatc tgctggtcct gctcttgggg atgcctctgg aaatctacga gatgtggcac     300
aattaccctt tcctgttcgg gcctgtggga tgctacttca agacagccct cttcgagact     360
gtgtgctttg cctccattct cagtgtcacc acggttagcg tagagcgcta tgtggccatt     420
gtccaccctt tccgagccaa gctggagagc acgcggcgac gggccctcag gatcctcagc     480
ctagtctgga gcttctctgt ggtcttttct ttgcccaata ccagcatcca tggcatcaag     540
ttccagcact ttcccaacgg gtcctccgta cctggctcag ccacctgcac agtcaccaaa     600
cccatgtggg tgtataactt gatcatccaa gctaccagct tcctcttcta catcctccca     660
atgaccctca tcagcgtcct ctactacctc atggggctca ggctgaagag agatgaatcc     720
cttgaggcga acaaagtggc tgtgaatatt cacagaccct ctagaaagtc agtcaccaag     780
atgctgtttg tcttggtcct cgtgtttgcc atctgctgga ccccccttcca tgtggaccgg     840
ctcttcttca gctttgtgga agagtggaca gagtccctgg ctgctgtgtt caacctcatc     900
catgtggtat caggtgtctt cttttatctg agctccgcgg tcaaccccat tatctataac     960
ctcctgtctc ggcgcttccg ggcggccttt cgaaatgttg tctcccctac ctgcaaatgg    1020
tgccatcccc ggcatcggcc acagggacct ccagcccaga agatcatctt cttgacagaa    1080
tgtcacctcg tggagctgac agaggatgca ggcccccagt tccctggtca gtcatccatc    1140
cacaacacca accttaccac ggcccccctgt gcaggagagg taccataa               1188
```

<210> 8
<211> 396

<212> PRT
<213> Rattus

<400> 8

```
Met Gly Lys Leu Glu Asn Ala Ser Trp Ile His Asp Pro Leu Met Lys
 1               5                  10                  15
Tyr Leu Asn Ser Thr Glu Glu Tyr Leu Ala His Leu Cys Gly Pro Lys
            20                  25                  30
Arg Ser Asp Leu Ser Leu Pro Val Ser Val Ala Tyr Ala Leu Ile Phe
        35                  40                  45
Leu Val Gly Val Met Gly Asn Leu Leu Val Cys Met Val Ile Val Arg
    50                  55                  60
His Gln Thr Leu Lys Thr Pro Thr Asn Tyr Tyr Leu Phe Ser Leu Ala
65                  70                  75                  80
Val Ser Asp Leu Leu Val Leu Leu Leu Gly Met Pro Leu Glu Ile Tyr
                85                  90                  95
Glu Met Trp His Asn Tyr Pro Phe Leu Phe Gly Pro Val Gly Cys Tyr
            100                 105                 110
Phe Lys Thr Ala Leu Phe Glu Thr Val Cys Phe Ala Ser Ile Leu Ser
        115                 120                 125
Val Thr Thr Val Ser Val Glu Arg Tyr Val Ala Ile Val His Pro Phe
    130                 135                 140
Arg Ala Lys Leu Glu Ser Thr Arg Arg Arg Ala Leu Arg Ile Leu Ser
145                 150                 155                 160
Leu Val Trp Ser Phe Ser Val Val Phe Ser Leu Pro Asn Thr Ser Ile
                165                 170                 175
His Gly Ile Lys Phe Gln His Phe Pro Asn Gly Ser Ser Val Pro Gly
            180                 185                 190
Ser Ala Thr Cys Thr Val Thr Lys Pro Met Trp Val Tyr Asn Leu Ile
        195                 200                 205
Ile Gln Ala Thr Ser Phe Leu Phe Tyr Ile Leu Pro Met Thr Leu Ile
    210                 215                 220
Ser Val Leu Tyr Tyr Leu Met Gly Leu Arg Leu Lys Arg Asp Glu Ser
225                 230                 235                 240
Leu Glu Ala Asn Lys Val Ala Val Asn Ile His Arg Pro Ser Arg Lys
                245                 250                 255
Ser Val Thr Lys Met Leu Phe Val Leu Val Leu Val Phe Ala Ile Cys
            260                 265                 270
Trp Thr Pro Phe His Val Asp Arg Leu Phe Phe Ser Phe Val Glu Glu
        275                 280                 285
Trp Thr Glu Ser Leu Ala Ala Val Phe Asn Leu Ile His Val Val Ser
    290                 295                 300
Gly Val Phe Phe Tyr Leu Ser Ser Ala Val Asn Pro Ile Ile Tyr Asn
305                 310                 315                 320
Leu Leu Ser Arg Arg Phe Arg Ala Ala Phe Arg Asn Val Val Ser Pro
                325                 330                 335
Thr Cys Lys Trp Cys His Pro Arg His Arg Pro Gln Gly Pro Pro Ala
            340                 345                 350
Gln Lys Ile Ile Phe Leu Thr Glu Cys His Leu Val Glu Leu Thr Glu
        355                 360                 365
Asp Ala Gly Pro Gln Phe Pro Gly Gln Ser Ser Ile His Asn Thr Asn
    370                 375                 380
Leu Thr Thr Ala Pro Cys Ala Gly Glu Val Pro Glu
385                 390                 395
```

<210> 9
<211> 25
<212> PRT
<213> Human

<400> 9

Phe Arg Val Asp Glu Glu Phe Gln Ser Pro Phe Ala Ser Gln Ser Arg
1 5 10 15
Gly Tyr Phe Leu Phe Arg Pro Arg Asn
20 25

<210> 10
<211> 23
<212> PRT
<213> Rat

<400> 10
Tyr Lys Val Asn Glu Tyr Gln Gly Pro Val Ala Pro Ser Gly Gly Phe
1 5 10 15
Phe Leu Phe Arg Pro Arg Asn
20

<210> 11
<211> 25
<212> PRT
<213> Pig

<400> 11
Phe Lys Val Asp Glu Glu Phe Gln Gly Pro Ile Ala Ser Gln Val Arg
1 5 10 15
Arg Tyr Phe Leu Phe Arg Pro Arg Asn
20 25

<210> 12
<211> 8
<212> PRT
<213> Pig

<400> 12
Tyr Phe Leu Phe Arg Pro Arg Asn
1 5

<210> 13
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR probe

<400> 13
gaaacagagc ctcgtacca 19

<210> 14
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR probe

<400> 14
agtcggatcc aattcaggtt ttgttaaagt gga 33

<210> 15
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR probe

<221> misc_feature
<222> (1)...(20)
<223> n = A,T,C or G

<400> 15
ttcagcctgg cngtntcnga 20

<210> 16
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR probe

<221> misc_feature
<222> (1)...(21)
<223> n = A,T,C or G

<400> 16
gctgaggatn gangcraarc a 21

<210> 17
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR probe

<400> 17
aggaaagggt aattgtgcca catctcgtag atttccagag gcatc 45

<210> 18
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR probe

<400> 18
cacagtctcg aagagggctg tcttgaagta gcatcccaca ggc 43

<210> 19
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe

<400> 19
ttctggtggt aatctttgag gcgatattgg cgtacctctg caagc 45

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/13386

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 14/72; C12N 15/16
US CL : 530/350; 514/2; 435/69.8, 7.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 514/2; 435/69.1, 69.8, 7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/22131 (BEHAN et al.) 20 April 2000, see SEQ ID NO: 11 and 12, and Examples 1(e), 3, 4, 6, and 7.	1-9, 13-25
X,P	HOWARD et al. Identification of receptors for neuromedin U and its role in feeding. Nature July 2000, Vol. 406, pages 70-74, see the entire document, especially Figures 1, 2, 5, and Table 1.	1-5, 10-25
X,P	RADDATZ et al. Identification and characterization of two neuromedin U receptors differentially expressed in peripheral tissues and the central nervous system. J. Biol. Chem. 20 October 2000, Vol. 275, No. 42, pages 32452-32459, especially Figure 2.	1-5, 13-25
Y,P	KOJIMA et al. Purification and identification of neuromedin U as an endogenous ligand for an orphan receptor GPR66 (FM3). Biochem. Biophys. Res. Comm. September 2000, Vol. 276, pages 435-438, entire document.	2-5, 10-12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
	27 AUG 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Eyvonne Eyler Telephone No. 703-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/13386

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/13386

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

Species I: SEQ ID NO:2,
Species II: SEQ ID NO:6.

The claims are deemed to correspond to the species listed above in the following manner:

Species I - claims 1, 16, 17, 24, and 25.
Species II - claims 1, 16, 17, 24, and 25.

The following claim(s) are generic: 2-15, 18-23, and 26.

The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:

Each species listed above has distinct chemical and structural properties, and therefore, these species do not share a special technical feature within the meaning of PCT Rule 13.2, and thus do not relate to a single invention concept within the meaning of PCT Rule 13.1.

Continuation of B. FIELDS SEARCHED Item 3:
STN (Medline, Biosis), EAST (patents)
Search terms: neuromedin U and Neuromedin U receptor